

## PATENT COOPERATION TREATY

PCT

## NOTIFICATION OF ELECTION

(PCT Rule 61.2)

From the INTERNATIONAL BUREAU

To:

Commissioner  
 US Department of Commerce  
 United States Patent and Trademark  
 Office, PCT  
 2011 South Clark Place Room  
 CP2/5C24  
 Arlington, VA 22202  
 ETATS-UNIS D'AMERIQUE  
 in its capacity as elected Office

<b>Date of mailing</b> (day/month/year) 13 September 2001 (13.09.01)	
<b>International application No.</b> PCT/US00/19980	<b>Applicant's or agent's file reference</b> PF-0721 PCT
<b>International filing date</b> (day/month/year) 20 July 2000 (20.07.00)	<b>Priority date</b> (day/month/year) 22 July 1999 (22.07.99)
<b>Applicant</b> TANG, Y., Tom et al	

1. The designated Office is hereby notified of its election made:

☒ in the demand filed with the International Preliminary Examining Authority on:

20 February 2001 (20.02.01)

☐ in a notice effecting later election filed with the International Bureau on:2. The election ☒ was☐ was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No.: (41-22) 740.14.35	Authorized officer R. Forax Telephone No.: (41-22) 338.83.38
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# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US 00/19980

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
  
Although claims 18, 21 and 24 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:  
  
invention 1: claims 1 to 27 partially

### Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

the 1990s, the number of people in the UK who are aged 65 and over has increased from 10.5 million to 12.5 million, and the number of people aged 75 and over has increased from 4.5 million to 6.5 million (Office of National Statistics 2000). The number of people aged 65 and over is projected to increase to 15.5 million by 2020, and the number of people aged 75 and over to 8.5 million (Office of National Statistics 2000). The increase in the number of people aged 65 and over is expected to be due to a combination of factors, including a decline in the birth rate, a decline in the death rate, and a decline in the rate of emigration.

The increase in the number of people aged 65 and over is expected to have a significant impact on the UK's health and social care system. The number of people aged 65 and over who are in need of health and social care services is expected to increase from 1.5 million in 1990 to 2.5 million in 2020 (Office of National Statistics 2000). This increase is expected to be due to a combination of factors, including a decline in the birth rate, a decline in the death rate, and a decline in the rate of emigration.

The increase in the number of people aged 65 and over is expected to have a significant impact on the UK's health and social care system. The number of people aged 65 and over who are in need of health and social care services is expected to increase from 1.5 million in 1990 to 2.5 million in 2020 (Office of National Statistics 2000). This increase is expected to be due to a combination of factors, including a decline in the birth rate, a decline in the death rate, and a decline in the rate of emigration.

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FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

Invention 1: Claims 1 to 27 partially

Isolated polypeptide comprising an amino acid sequence of SEQ ID NO:1 and polynucleotide of SEQ ID NO: 16, biologically active and immunogenic fragments thereof, antibodies which specifically bind thereto, composition comprising said polypeptide and methods of using said polynucleotides and polypeptides

Inventions 2 to 12: Claims 1 to 27 partially

Isolated polypeptide comprising an amino acid sequences of SEQ ID NO:2 and 4 to 14 and polynucleotides of SEQ ID NO: 17 and 19 to 29, biologically active and immunogenic fragments thereof, antibodies which specifically bind thereto, composition comprising said polypeptide and methods of using said polynucleotides and polypeptides



# INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter: Application No

PCT/US 00/19980

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9816648	A	23-04-1998	EP 0932685 A	04-08-1999
WO 9740174	A	30-10-1997	NONE	
US 5759833	A	02-06-1998	NONE	



## INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference <b>PF-0721 PCT</b>	<b>FOR FURTHER ACTION</b> see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
International application No. <b>PCT/US 00/ 19980</b>	International filing date (day/month/year) <b>20/07/2000</b>	(Earliest) Priority Date (day/month/year) <b>22/07/1999</b>
Applicant <b>INCYTE GENOMICS, INC.</b>		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 6 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

**1. Basis of the report**

- a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

- b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

☒ contained in the international application in written form.

☒ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. ☒ **Certain claims were found unsearchable** (See Box I).

3. ☒ **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No.

☐ as suggested by the applicant.

☐ because the applicant failed to suggest a figure.

☐ because this figure better characterizes the invention.

☒ None of the figures.



## INTERNATIO SEARCH REPORT

International application No.  
PCT/US 00/19980**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
  
Although claims 18, 21 and 24 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:  
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3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

invention 1: claims 1 to 27 partially

**Remark on Protest**

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.





**FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210**

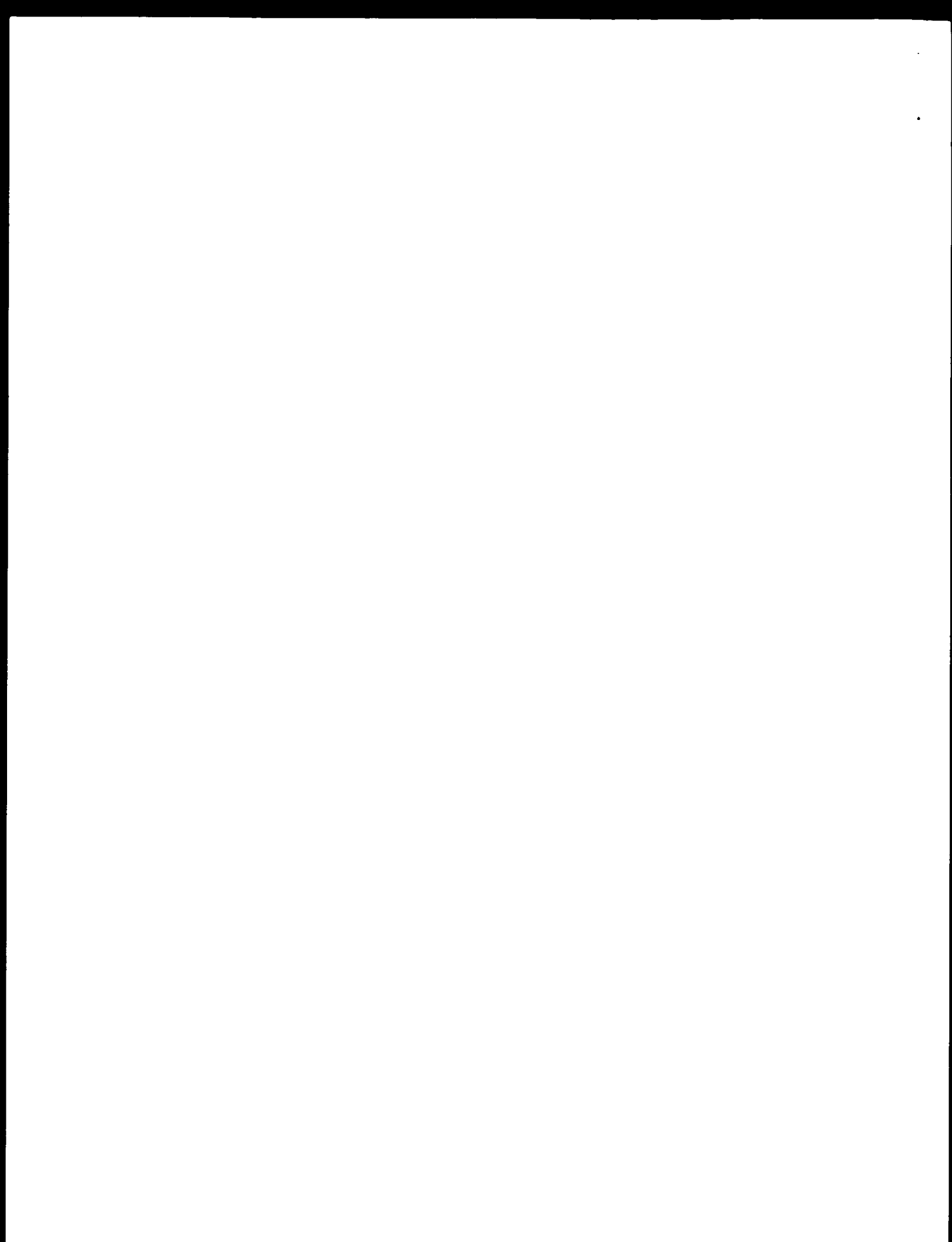
This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

**Invention 1: Claims 1 to 27 partially**

Isolated polypeptide comprising an amino acid sequence of SEQ ID NO:1 and polynucleotide of SEQ ID NO: 16, biologically active and immunogenic fragments thereof, antibodies which specifically bind thereto, composition comprising said polypeptide and methods of using said polynucleotides and polypeptides

**Inventions 2 to 12: Claims 1 to 27 partially**

Isolated polypeptide comprising an amino acid sequences of SEQ ID NO:2 and 4 to 14 and polynucleotides of SEQ ID NO: 17 and 19 to 29, biologically active and immunogenic fragments thereof, antibodies which specifically bind thereto, composition comprising said polypeptide and methods of using said polynucleotides and polypeptides



## INTERNATIONAL SEARCH REPORT

International Application No

US 00/19980

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/52 C12N9/00 C07K16/40 C12Q1/68 A61K38/43  
A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N C07K C12Q A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

STRAND, EPO-Internal, PAJ, WPI Data

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	NAGASE T ET AL: "PREDICTION OF THE CODING SEQUENCES OF UNIDENTIFIED HUMAN GENES. XVITHE COMPLETE SEQUENCES OF 150 NEW CDNA CLONES FROM BRAIN WHICH CODEFOR LARGE PROTEINS IN VITRO" DNA RESEARCH, UNIVERSAL ACADEMY PRESS, JP, vol. 7, 2000, pages 65-73, XP000949814 ISSN: 1340-2838 SEQUENCE KIAA1352	1-17
P,X	--- DATABASE EBI [Online] SEQUENCE AC D84223, 15 May 2000 (2000-05-15) H. MOTEGI ET AL.: "Cloning and sequence determination of a human cytoplasmic leucyl-tRNA synthetase gene." XP002152778 sequence data --- -/-	1-17

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

## \* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&amp;" document member of the same patent family

Date of the actual completion of the international search

14 November 2000

Date of mailing of the international search report

19. 02. 01

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
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Authorized officer

Hix, R



## INTERNATIONAL SEARCH REPORT

International Application No

US 00/19980

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 98 16648 A (BALDWIN JACK EDWARD ;CLIFTON IAN (GB); ISIS INNOVATION (GB); ROACH) 23 April 1998 (1998-04-23) the whole document ---	
A	WO 97 40174 A (LEUKOSITE INC) 30 October 1997 (1997-10-30) the whole document ---	
A	US 5 759 833 A (SHIBA KIYOTAKA ET AL) 2 June 1998 (1998-06-02) the whole document -----	

The first part of the document discusses the importance of maintaining accurate records of all transactions. It emphasizes that proper record-keeping is essential for ensuring the integrity of the financial system and for providing a clear audit trail. The document also highlights the need for transparency and accountability in all financial dealings.

In the second part, the document outlines the various methods used to collect and analyze data. It describes the process of gathering information from different sources and how this data is then used to identify trends and patterns. The document also discusses the importance of using reliable and valid data sources to ensure the accuracy of the findings.

The third part of the document focuses on the results of the analysis. It presents the findings of the study and discusses the implications of these results. The document also provides recommendations for future research and for the implementation of the findings in practice.

Finally, the document concludes by summarizing the key points and reiterating the importance of the research. It emphasizes that the findings of the study have significant implications for the field and that further research is needed to fully understand the issues at hand.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

US 00/19980

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9816648	A	23-04-1998	EP 0932685 A	04-08-1999
WO 9740174	A	30-10-1997	NONE	
US 5759833	A	02-06-1998	NONE	





<b>(51) International Patent Classification <sup>6</sup> :</b> <b>B65D 51/24, 51/28, 77/24</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 97/19001</b>
		<b>(43) International Publication Date:</b> 29 May 1997 (29.05.97)

(74) Agent: LANGFELDT, Jens, F., C.; Bryns Patentkontor A/S,  
P.O. Box 765, Sentrum, N-0106 Oslo (NO).

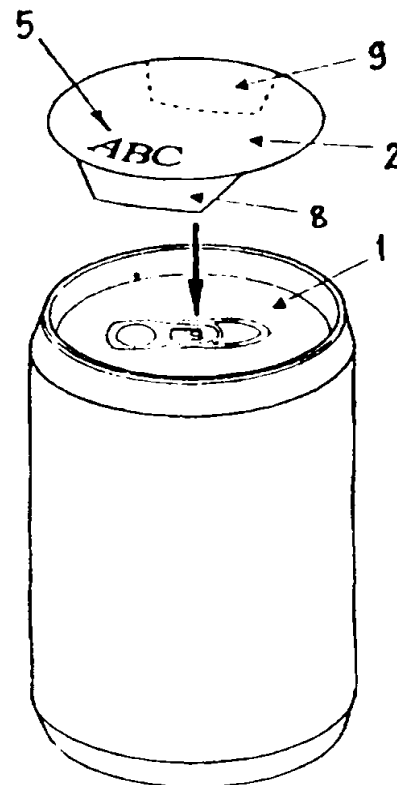
(11) International Publication Number: WO 97/19001  
(43) International Publication Date: 29 May 1997 (29.05.97)

**(81) Designated States:** AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

*In English translation (filed in Norwegian).*

**(57) Abstract**

A device for a beverage can, wherein the top (1') of the container (1) has a depressed portion (6), and wherein the top surface (3') of the can has a detachable section (4) connected to a pouring aperture generating pull means (3). The depressed portion interacts with a cover (2, 13; 13'; 20), optionally designed to have or to form a cavity. Said cavity is capable of accommodating an article or a foodstuff. The cover, optionally its effected cavity, may in addition be provided with legible information, e.g., an advertisement.



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Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

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GA	Gabon			VN	Viet Nam

## 1

The present invention relates to a device for a beverage can as disclosed in the preamble of the attached patents claims 1, 2 and 3. Furthermore, the invention relates to a can of  
5 a stackable type as disclosed in claim 14.

The point of departure for the present invention is to provide in connection with beverage cans a best possible covering of the top and/or bottom of the can, so that there is no likelihood of primarily the top part of the can becoming contaminated with dirt, dust or the like around the pouring aperture of the can, whilst ensuring that the can is capable of being stacked, and also that use of the external bottom area of the can is possible.

From US Patent 5,131,554 it is known to provide a beverage can with a dust cover which is riveted to the pull means which is to be used to tear open the pouring aperture. The cover is thus designed to lie completely flush with the top of the can and requires a can type which is not conventionally used.

US Patent 5,273,176 relates to a solution where a cover capable of being pivoted upwards can be provided over the top of the can, and where the edges of the cover extend beyond the periphery of the can top. The cover has a through-going hole for a straw and is designed to be positioned such that relative to the pouring aperture of the can, said hole in the cover corresponds to the pouring aperture when this has been opened. The object of the taught solution is also to be able to close the cover after drinking from the can with the aid of the straw.

A disadvantage of this known solution is that the stackability of the cans is affected, whilst arrangement in connection with, e.g., a six-pack, is rendered virtually impossible owing to the reduced attachment flange on the can.

- 5 US Patent 5,139,163 relates to a solution reminiscent of that taught in US Patent 5,273,176. In this known solution, clustering, e.g., a six-pack, is rendered completely impossible owing to the fact that the cover rests on the outside of the can top and the top flange of the can is no longer accessible. The cover is provided with a tear-off means to make the pouring aperture of the can and its pull means accessible. Just as in the other
- 10 known solutions, a proportional, mutual orientation between the positioned cover and the pouring aperture of the can is required if the user is to enjoy the benefits the cover is intended to give. However, a solution of this kind is difficult to combine with an automated process.
- 15 US Patent 4,708,257 describes a beverage can with a seal which is adhered or shrunk onto the top of the can to prevent the top of the can from being contaminated. The seal may consist of a circular, thin film or foil which curls up where it is torn open from one side.
- 20 US Patent 4,651,890 makes known a solution wherein on the underside of the can there is provided a tear-open pouch containing a moist wipe means for cleaning the top of the beverage can prior to drinking directly from the can.
- US Patent 1,879,517 relates to a can, jar or the like, where the top or bottom of the can
- 25 is provided with a further lid or cover in addition to the real lid and cover, and where this additional lid or cover may contain an article of some kind.
- US Patent 3,638,787 relates to a special package for packing several cans together in addition to a fragile unit of some kind which is not a part of the actual cans, but is
- 30 hidden by the packaging. This solution is particularly well suited in connection with packing fragile articles which cannot be packed mechanically.
- US Patent 3,112,824 relates to a can or bottle wherein the top of the can or bottle is provided with a cover which may contain an article of some kind.
- 35 US Patent 4,917,258 relates to a lid which can be snapped on to a beverage can.

US Patent 2.804,201 relates to a solution whereby a lid can be placed on the top of a can which incorporates a can opener key, and where the lid when turned can be brought into engagement with the handle of the can opener key.

- 5 US Patent 2,120,403 relates to a solution where some form of advertising matter may be provided on the actual can opener key which is secured in a conventional manner to the top of the can, whereupon the top of the can may be covered with a lid. This lid is shown having various types of design where it is possible to allow the space between the lid and the cavity of the can to contain an article of some kind.

10

European Patent Publication 404 734 shows a further illustration of the state of the art, where the edge of a beverage can may be provided with a snap-on drinking lip so that the person drinking from the can does not come into direct contact with the metal of the beverage can, whilst the device also enables a certain covering of the exposed aperture  
15 leading into the cavity of the can, which may be practical if there is a desire to temporarily close the can.

- An object of the present invention is to provide a solution in connection with a beverage can, whereby the cover which is to be arranged on the top surface of the can is capable  
20 of being attached in a simple manner.

The characterising features of the aforementioned devices and the can of a stackable type are set forth in the claims below.

- 25 However, an object of the present invention is to provide solutions where a covering of the pouring aperture of the can may be combined with other advantages, whilst at the same time other possibilities have been discovered with the present invention for the use of surfaces on a beverage can of this kind.

- 30 The invention will now be described in more detail with reference to the attached drawings.

Figs. 1 to 5 illustrate a first embodiment of the device according to the invention.

- 35 Figs. 6 and 7 illustrate a second embodiment of the device according to the invention, with different types of joints between the cover and the beverage can.

Figs. 8 and 9 illustrate a third embodiment of the device according to the invention, with different types of joints between the cover and the beverage can.

5 Figs. 10, 11, 12 and 13 show alternative methods of securing cans in clusters for the embodiment according to Fig. 8.

Figs. 14 and 15 show a third embodiment of the device according to the invention and represent a modification of the solution in Figs. 9 and 13.

10 Figs. 16 to 18 illustrate a fourth embodiment of the device according to the invention.

Figs. 19 to 21 show a fifth embodiment of the device according to the invention.

15 In Fig. 1 it is shown how a beverage can 1 may be provided with a cover 2. The can 1 has a pull means 3 which actuates a weakening 4 in the top of the can in order to generate a pouring aperture.

The cover 2 may, e.g., be dome-shaped, as indicated in Fig. 5. The cover 2 may be provided with print 5, e.g. a printed advertisement, indicated as a mere example in the form of the letters ABC. However, it will be understood that any form of print is possible. Alternatively the print could be in the form of impressions in the material of the cover, e.g., produced during the forming process of the cover. The cover could be wholly or partly transparent, but it is also conceivable that cover is opaque in that a coating is applied, e.g., in the form of a protecting lacquer. Furthermore, it is also possible to allow the cover 2 to be light-reflecting perhaps in order to draw better attention to the message provided on the cover. As can be seen from Fig. 1, the cover 2 is designed to fit into the depressed portion of the container top, so that the wall 6 of the depressed portion substantially bears against the periphery of the cover, as can better be understood with reference to Fig. 5.

30 The cover 2 will effectively protect the pouring aperture of the can from dust and contaminants before the can is emptied.

35 The attachment system of the cover which is shown in Figs. 1 to 5 is particularly useful for those cases where the cover is only to be provided with print, e.g., a printed advertisement or is made having print in the form of impressions in the material of the cover. In this case the cover is preferably opaque. As can be seen from Fig. 4 the cover

is made in this case of a sheet part 7 which has two diagonally arranged, backwardly slanting, opposing fixing flaps 8 and 9.. These fixing flaps are designed such that when the cover 2 is pressed down towards the can top 3', the flaps 8 and 9 are bent in the direction of the front face of the cover 2 or the sheet part 7 as indicated by means of the arrows 10, whereby the fixing flaps come into engagement between the top surface 3' of the can and the said pull means 3 in connection with the pouring aperture 4. The flaps preferably have an angle of departure in the range of 40-50°, although this range must not be seen to be limiting for the present invention. As shown in Fig. 5, when the cover 2 is in place, the top part 7 thereof will be slightly curved forwards or upwards, whilst the flaps 8 and 9 remain substantially parallel to the surface 3' of the can top. In this way an effective attachment method of the cover on to the top of a beverage can is obtained. An advantage of the solution shown in Figs. 1 to 5 is that engagement will take place with the pull means 3 irrespective of the mutual orientation of the fixing flaps 21 and 22 relative to the orientation of the pull means 3. This attachment method which can thus be seen from Figs. 1 to 5 is particularly suitable for automated application of the cover 2. The cover 2 will effectively be under tension when it is arranged in the position shown in Fig. 5.

A further development of the invention is also shown in Figs 6 to 9 where the cover is designed as a cavity, the cover in reality being made having an upper portion 11 and a lower portion 12 which in essence correspond to the design of the upper portion 1' and the lower portion 1'' of the beverage can 1. However, it can readily be envisaged that the depressed portions in the upper 11 and lower 12 portions of the cover respectively may be of a depth that is less than that of the depressed portions in the upper 1' and lower 1'' portions of the can. The cover in the present case is indicated by means of the reference numeral 13. The cover has a tear-off portion 4'. The cover, which in terms of appearance resembles a beverage can, has however substantially smaller axial extent L2 than the axial extent L1 of the beverage can. As a, for the invention non-limiting, example, the cover 13 may have an axial length which is equal to about 20 to 50% of the axial extent of the beverage can 1. The cover 13 in the form of a mini-can may be secured to the beverage can, e.g., by adhesion, as shown in Figs. 6 and 8. In the embodiment in Fig. 6, this takes place in that the lower, bevelled portion 12 of the cover 13 is provided with an adhesive, whilst in the embodiment in Fig. 8 the lower bevelled portion 14 on the beverage can is provided with an adhesive. In the embodiments according to Figs. 7 and 9, the cover part 13 is attached to the beverage can by means of tape or shrink wrap plastics 16.

As can be seen in Fig. 10, an assembled unit consisting of a can 1 and a bottom cover 13 could form a part of a multi-pack, e.g., a six-pack. The six assembled can units consisting of three cans 1 and three bottom covers 13 are retained in a known way per se by a plastic film 15 known per se that is provided with holes and where the edges of the holes form secure engagement with the lower edge of the folded flange of the can.

In Fig. 11 an alternative packing form is shown, e.g., of four units 1, 13, wherein a packing plastic film 17 extends around the top of the uppermost unit and around the bottom of the lowermost unit. The plastic film may optionally be of a type such that it has a carrying handle 18. It would optionally be expedient to connect the respective parts 1 and 13 in each unit with an adhesive to facilitate the packing. However, this is not a definite condition for the understanding and performance of the present invention.

In Fig. 12 it is shown that the container 13 may instead form a top cover 13' for the can 1. In this case it would be expedient to join the cover 13' and the can 1 with an adhesive, optionally to pack the units together in the same way as shown in connection with Fig. 11. In case the cover 13' is designed to be identical to the cover or mini-can 13 which is shown in connection with Figs. 6 to 9, and where the mini-can 13' is adhered to the beverage can 1, a six-pack, for example, may be held together by means of a supporting plastic film 19 in a manner corresponding to that shown and described in connection with Fig. 10. However, it will be understood that other packing methods will be conceivable within the scope of the invention.

In this connection reference can also be made to Fig. 13 where three units which each consist of a can 1 and a cover 13 are arranged in a column, and where the joining takes place with the aid of tape or shrink wrap plastic 16 in the transition portions between the units.

As an example of the content in a cover or mini-can 13 or 13', mention can be made of articles in solid or liquid form, including typical taste samples such as introductory samples of new products, peanuts, chocolate and other foodstuffs or stimulants. It will immediately be appreciated that the covers represented by the mini-cans 13 and 13' will be capable of having print applied, e.g., a printed advertisement both around the periphery and on the top and bottom surfaces.

In Fig. 14 a solution is shown with a cover 20 which has a recess 21 which is adapted to the external periphery or bevelled portion 14 of the can 1, and on its other side has an



axially projecting integrated body 22 with a periphery adapted to the inner wall 6 in the depressed portion on an adjacent beverage can 1. However, it is worth noting that the cover 20 has a cross-section D2 which is no greater than the cross-section D1 of the beverage can. The cover 20 in the illustrated embodiment is intended to constitute a supporting mat for the can, e.g., made of a cork material or other material which does not scratch the underlying surface on which the beverage can 1 is to be placed.

Between the depressed portion 21 and the recess in the bottom of the can there may be placed an article, e.g., selected from a group consisting of lapel badges, mini-art, mini-CDs, lottery tickets, printed matter, mini-toys, souvenirs, bonus coupons, samples, serviettes, wipes, taste samples, advertisements, change. The use of the said space or cavity for change may be particularly relevant in connection with the sale of beverage cans or bottles in vending machines.

As an alternative to cork material, the cover 20 may be made of, for example, a soft plastic material or another material which under normal conditions does not cause scratching. Optionally, the cover 20 may conceivably be made of a wood fibre material.

To be able to sell the solution shown in Fig. 14 in an expedient manner, tape or shrink plastic 23 could conceivably be used to attach the cover 20 to the beverage can 1. However, this does not rule out the use of dots of adhesive between the can 1 and the cover 20. The sales unit as shown in Fig. 15 consists of a total of three can/cover units 1, 20. The transitions between these units are made with the aid of tape or shrink wrap plastics. Alternatively, the connection can be made as shown and explained in connection with Fig. 11.

Although the said covers are preferably made of the same material as the beverage can, e.g., aluminium, this does not, of course, prevent other materials from being chosen for use in the covers. Possible alternative materials may conceivably be rigid or soft thermoplastics, or wood fibre materials, materials containing corn starch or other starch products, and optionally appropriate shrink wrap plastic materials. However, it should be noted that the choice of material for the covers should in no way be seen as limiting for the idea and scope of the invention.

Although in connection with the said covers, no consideration has been given to a direct covering of the top and outer edge of the can, there is of course nothing to prevent the top portion from being provided with a protecting film, e.g., of aluminium or plastic.

However, when the can assembly is supplied in the form illustrated, e.g. in Figs. 13 and 15 and with the modifications thereof that are obvious to the skilled person, it will immediately be appreciated that a hygienic protection of the drinking edge of the beverage can could also be provided in part by those tape or shrink wrap plastic materials which are arranged around the transitions between the can/cover unit and an adjacent unit of this kind. The carrying piece which is located on the top of such a unit could easily be made so as to provide a sufficient covering of the top edge of the upper can 1. In the solution shown in Fig. 11, it will be ensured that the drinking edge is kept clean by using shrink plastic between the can 1 and the cover 13' for each unit thereof.

10 If the mini-can 13' in addition has an upper portion which is equipped with a folded flange, it is possible to move the connecting film up to this portion of the can 13'.

In the embodiment according to Figs. 16 to 18, the cover 24 is intended for a beverage can 25, preferably of the stackable type, wherein the top of the can has a depressed portion 26 with a further recessed rim along the inner wall 28 of the depressed portion.

15 As previously described, the can is in a known way designed to have a detachable section connected to a pouring aperture generating pull means 30.

The cover is designed to fit into the depressed portion 26 and has a cavity 24' or a recess in its back. The wall 28 of the depressed portion surrounds and substantially bears against the periphery of the cover over at least a part of its axial extent.

20

This alternative solution is characterised in that the cover 24 along upper outer rim thereof has a sealing lip 31 designed to bear against said inner wall 28 and along its lower outer rim has a bead 32 designed to fit into said recessed rim 27. When the cover 24 is pressed down into the depressed portion 26 of the can and the rim 27, in the same operation there is also produced a vacuum between the back of the cover 24 and the top surface 26' of the can through vacuum actuation during the pressing action. The bead 32 is to advantage made of a material which, when the cover 24 is pressed down into the depressed portion 26 and vacuum is actuated simultaneously, is subjected to a lateral expansion into the recessed rim 27, thereby obtaining improved adhesion and sealing, so that said vacuum between the cover 24 and the depressed portion 26, 37 of the can is maintained. Furthermore, the recessed rim 27 can be provided with an adhesive 34 in order to further secure said vacuum through increased adhesion between the rim 27 and the bead 32 of the cover.

25

30

35

The cover is provided with a tear-off flap 33, which causes said vacuum between the cover 24 and the depressed portion 26, 27 of the can to be eliminated when the flap is torn off, whereby the bead 32 is released from the recessed rim 27, allowing the cover 24 and its optional content (not shown) to be removed.

5

Thus, the present invention does not only make possible the covering of the pouring aperture of a beverage can, and the application of advertising or information on a cover which is located on the top or at the bottom of the beverage can 1 or in both places, but also allows the said covers to contain objects or samples in liquid or solid form. When  
10 supplying units in, e.g., three-packs, four-packs, six-packs or the like, it will be understood that it will be possible to attach beverage cans 1 and their respective covers 13, 13' to one another in a simple manner and also in this way ensure that the drinking edge is either covered or optionally can be cleaned with a wipe which is found in the cavity or recess of the cover.

15

In the solution shown in Figs. 19-21, the cover 13 is arranged to cover the depressed bottom portion 37 of the can. However, the cover 13 may conceivably instead cover the depressed top portion 6 of the can, as indicated in Fig. 7. The assembly of the can (1) and cover (13) is then surrounded by an upwardly open container 35, preferably made in  
20 the form of a drinking glass of plastic or glass. The drinking glass may be made of a transparent, translucent or opaque material. Like the can, the outer surface of the glass may optionally be provided with decoration, ornament, information or advertising material.

25 However, it is important that when storing the can 1, the cover 13, and the glass 35 that dust and dirt do not enter the container in the space between the can/cover assembly and the inner wall of the container 35. Therefore, around a upper portion 35' of the container 35 there is provided a sealing means 36 which is detachable, e.g., by tearing off. The sealing means may, e.g., be a band of heat shrinkable material, a sealing  
30 sleeve, a sealing tape, e.g., an adhesive tape. The sealing means 36 rests in sealing contact with a peripheral portion 1''' of the can 1/cover 13 assembly, e.g., a portion 1''' of the can as indicated in Fig. 20.

Although it is not shown in Figs. 19-21, it will be understood that a type of cover as  
35 shown and described in connection with Figs. 1 to 15 or Figs. 16 to 18 could be provided in the recessed portion 6 of the can 1 or in the recessed portion 38 of the cover

13 depending upon whether it is the can 1 or the cover 13 which is uppermost in the can 1/cover 13 assembly.

The solution shown in Fig. 19 is particularly advantageous in the cases where in a practical manner it is desirable to sell or distribute as a unit a can 1 containing a  
5 beverage, cover 13 containing another beverage or a foodstuff in solid form, e.g., peanuts, and a drinking glass 35.

Patent claims

1.

- 5 A device for a beverage can, preferably of a stackable type, wherein the bottom and/or top of the can (1) has a depressed portion, wherein the top of the can has a detachable section (4) connected to a pouring aperture generating pull means (3), where in the depressed portion there is provided a cover (2) which is designed as a cavity, or which on its back has a recess, and where the wall of the depressed portion surrounds and
- 10 substantially bears against the periphery of the cover over at least a part of its axial extent, characterised in that the cover (2) on the back thereof is provided with two diagonally arranged, backwardly inclined, opposing fixing flaps (8, 9) and designed, when the cover (2) is pressed down towards the top of the can (3') thereby bending the fixing flaps in the direction of the front of the cover, to come into engagement between
- 15 the top surface of the can and said pull means (3).

2.

- A device for a beverage can, preferably of a stackable type, wherein the bottom and/or top of the can (1) has a depressed portion, and wherein the can top has a detachable
- 20 section (4) connected to a pouring aperture generating pull means (3), characterised by a cover (13; 13'; 20) having an upper and a lower portion which essentially correspond to the top portion and the bottom portion of the beverage can (1), and designed to cover the depressed portion of the can bottom or top, and where the cover has an axial extent (L2) which is substantially smaller than the axial extent (L1) of the beverage can.

25

3.

- A device for a beverage can, preferably of a stackable type, wherein the top of the can has a depressed portion (26) with a further recessed rim (27) along the inner wall (28) of the depressed portion, and also a detachable section (29) connected to a pouring aperture
- 30 generating pull means (30), wherein in the depressed portion there is provided a cover (24) which is designed to have a cavity (24') or on its back has a recess, and where the wall (28) of the depressed portion surrounds and substantially bears against the periphery of the cover over at least a part of its axial extent, characterised in that the cover (24) along its upper outer rim has a sealing lip (31) intended for contact with said
- 35 inner wall (28) and along its lower outer rim has a bead (32) designed to fit into said recessed rim (27), a vacuum being produced between the back of the cover and the top surface of the can, by arranging the cover (24) in the depressed portion (26) of the can.

4.

A device as disclosed in claim 3, characterised in that the bead (32) is made of a material which, when the cover is pressed down into the depressed portion (26) and vacuum is actuated simultaneously, is caused to expand laterally into the recessed rim (27).

5.

10 A device as disclosed in claim 3 or claim 4, characterised in that in the recessed rim (27) there is provided an adhesive (34) for adhesion to the bead (32) of the cover (24).

6.

A device as disclosed in claims 2, 3 or 4, characterised in that the cover (13; 13') has a tear-off portion (4').

7.

A device as disclosed in claim 2, characterised in that the cover (13; 13'; 20) is secured to the can (1) with the aid of an adhesive or wrapping material.

20 8.

A device as disclosed in claim 2, 6 or 7, characterised in that the axial extent (L2) of the cover (13; 13'; 20) is 20-50% of the axial extent (L1) of the beverage can.

9.

25 A device as disclosed in one or more of claims 2, 6-8, characterised in that the cover (13; 13'; 20) is adapted to contain a foodstuff or stimulant in liquid or solid form.

10.

A device as disclosed in one or more of claims 2 to 9, characterised in that the cover (13; 13'; 20) is adapted to contain an article.

11.

35 A device as disclosed in one or more of claims 2, 6, 7 or 8, characterised in that the cover (13) is arranged to cover the depressed portion of the can (1), and that the assembly of the can (1) and the cover is surrounded wholly or partly by an upwardly open container (35).

12.

A device as disclosed in claim 11, characterised in that about the upper portion (35') of the container (35) there is provided a detachable sealing means (36), e.g., a band of heat shrinkable material, a sealing sleeve or sealing tape, which is in sealing contact with a  
5 peripheral portion (1'') of said assembly, e.g., a portion of the can (1) or a portion of the cover (13).

13.

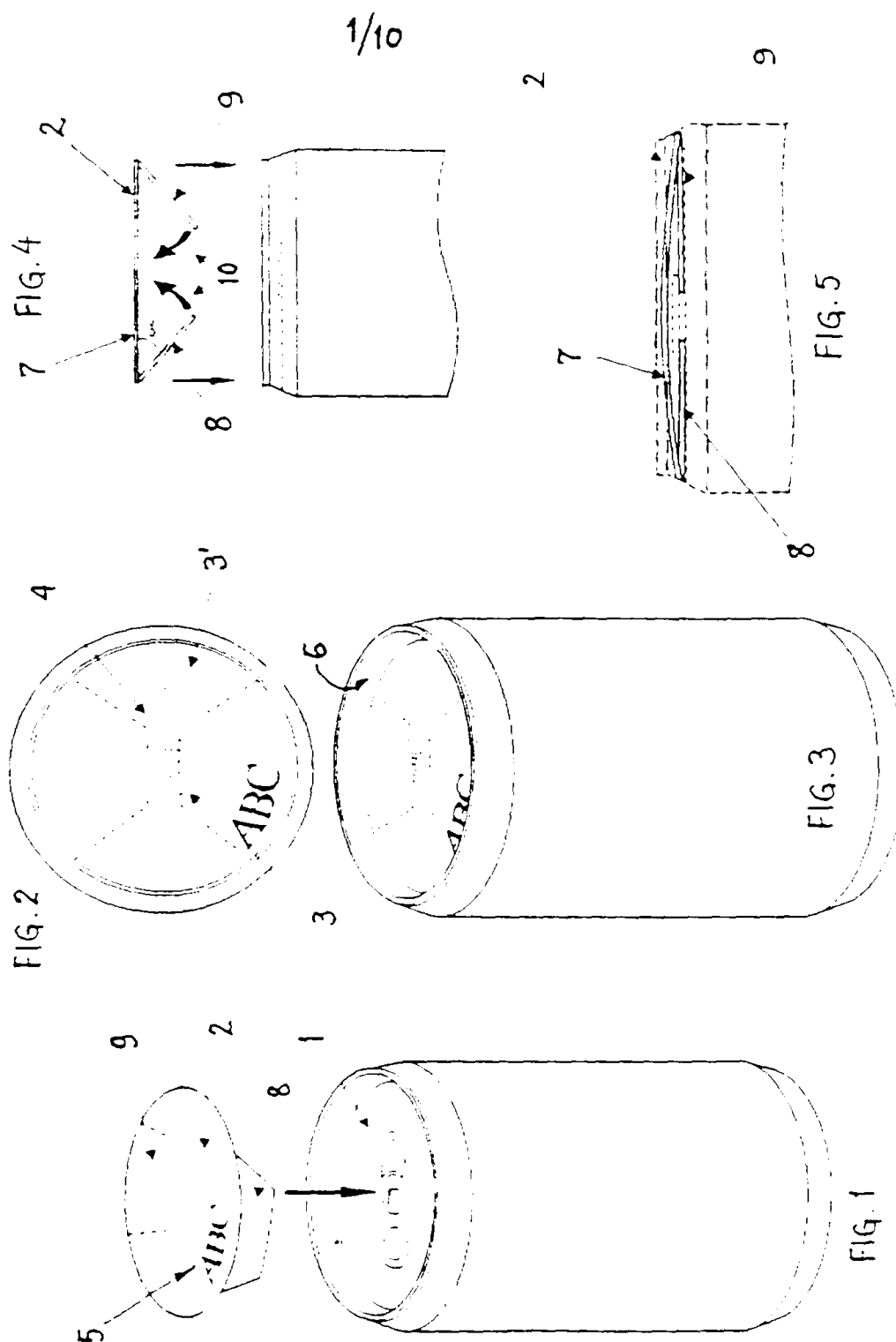
A device as disclosed in claim 11 or 12, characterised in that the container (35) is made  
10 in the form of a drinking glass.

14.

A can of a stackable type, wherein the bottom and/or top of the container has a depressed portion and is provided uppermost and lowermost with an inwardly curved  
15 end portion to enable the container to be stacked with a can located above and/or below, made having a substantially similar bottom and top portion, and where the top surface of the can has a detachable portion (4') connected to a pouring aperture generating pull means (3), characterised in that the can (13, 13') has an axial extent which is  
20 substantially smaller than the axial extent of a known beverage can (1) per se, preferably 20-50% of the axial extent of the beverage can (1), and that it serves as a detachable cover for the top or bottom of such a known beverage can (1) per se.

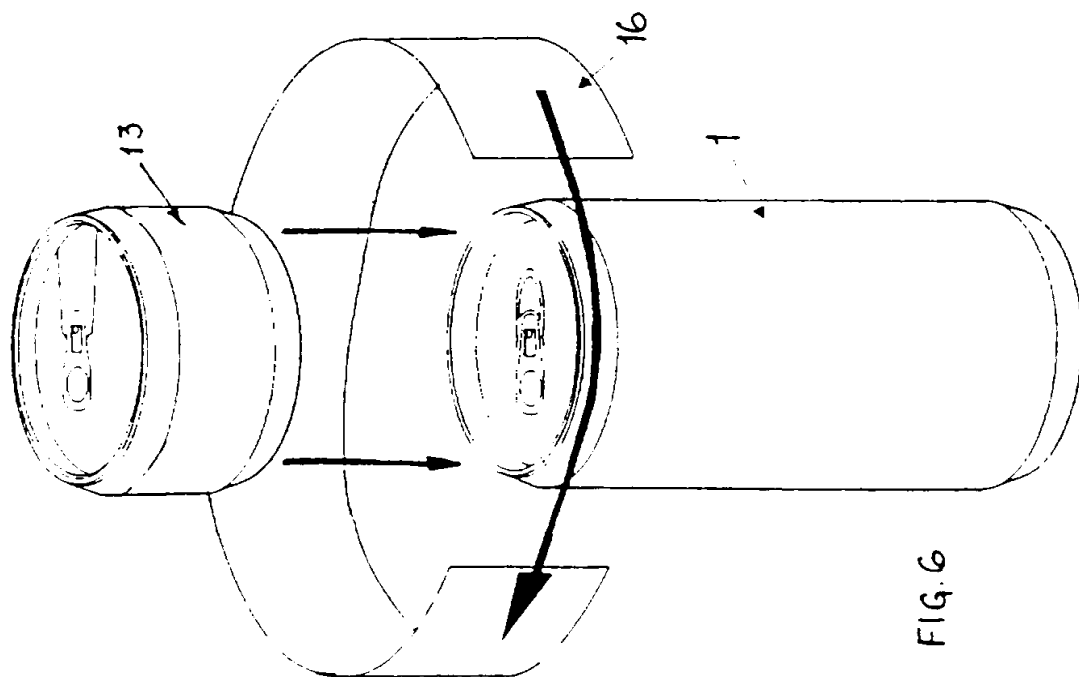
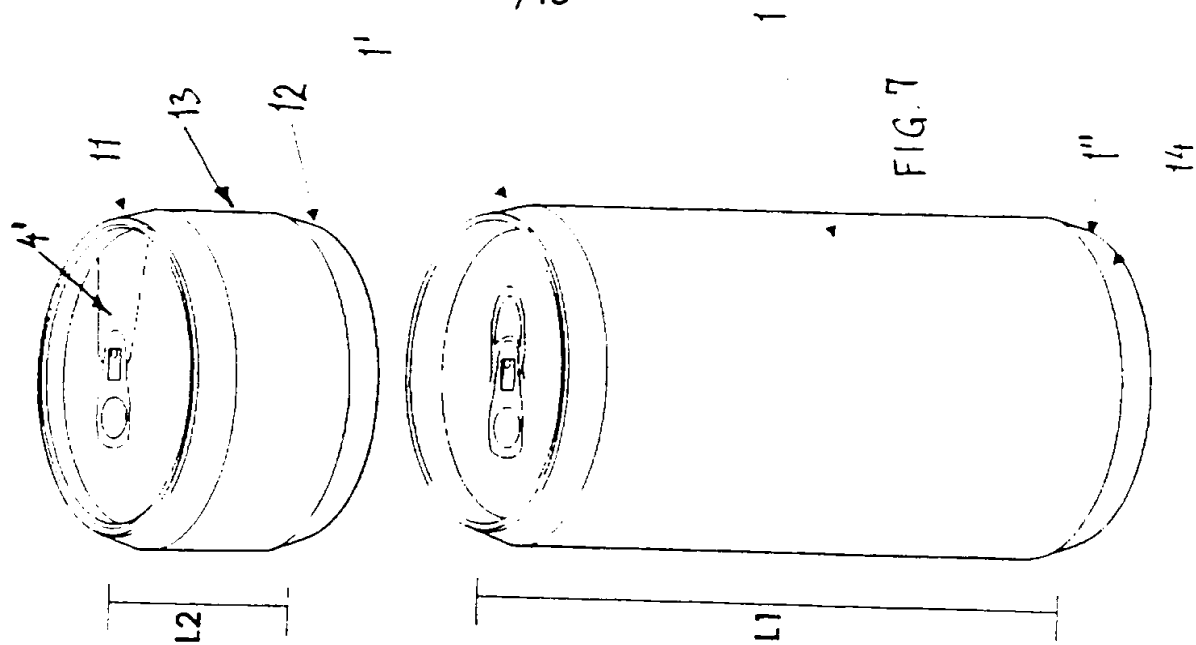
15.

A can as disclosed in claim 14, characterised in that it contains a foodstuff or stimulant  
25 in liquid or solid form, or an article.





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3/10

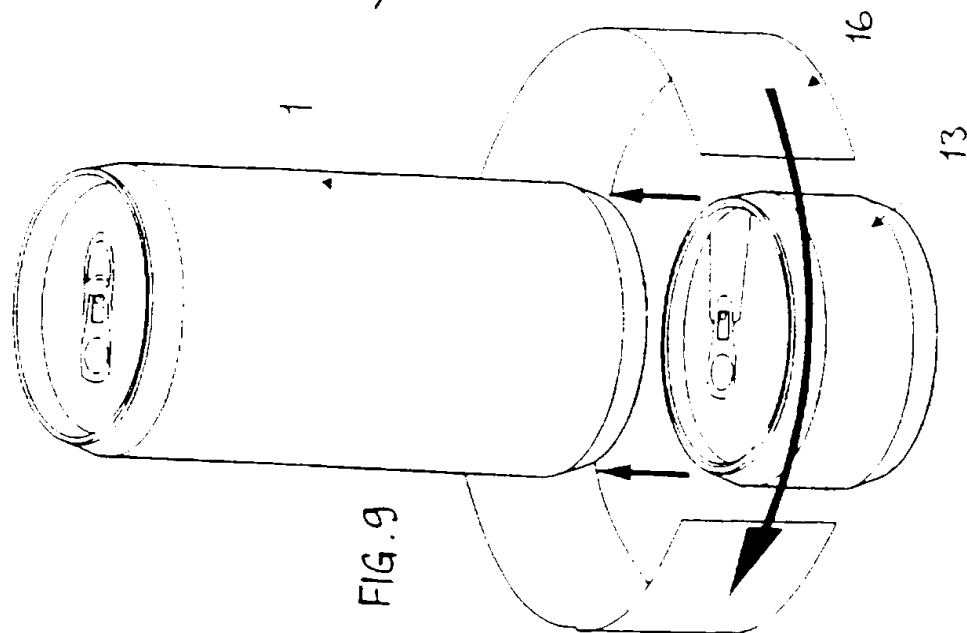


FIG. 9

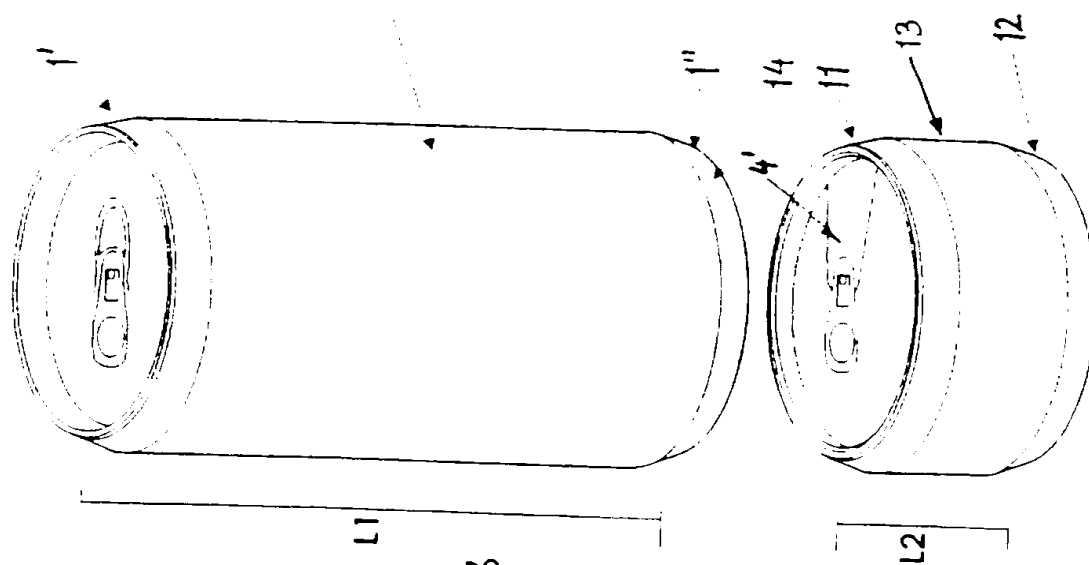


FIG. 8

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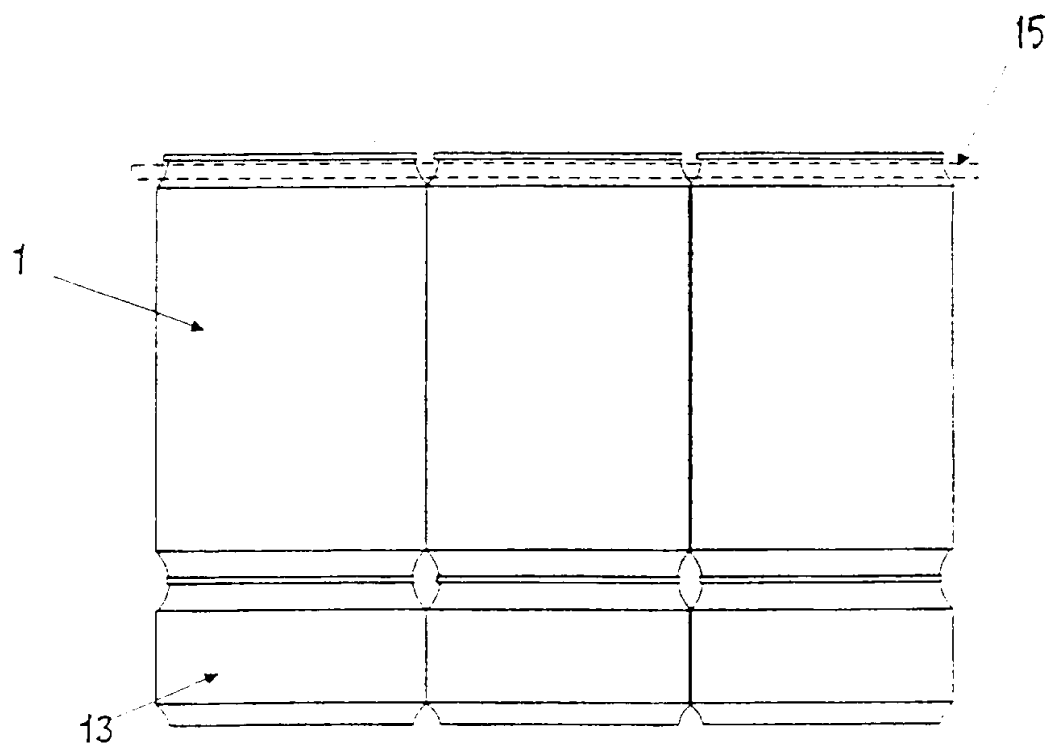


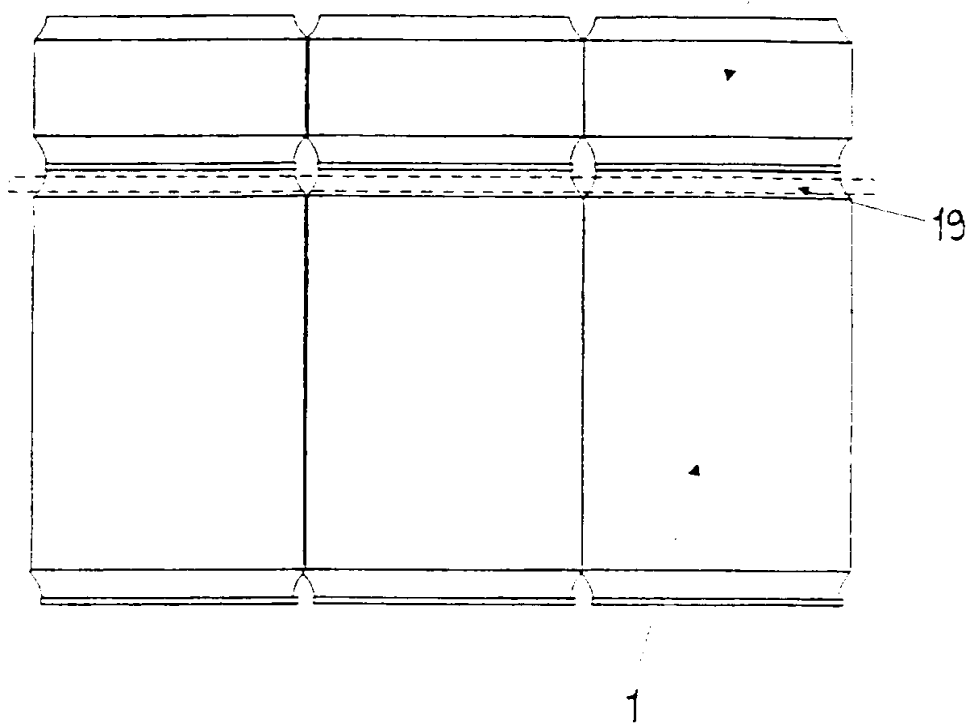
FIG. 10



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FIG. 12

13'





8/10

FIG. 14

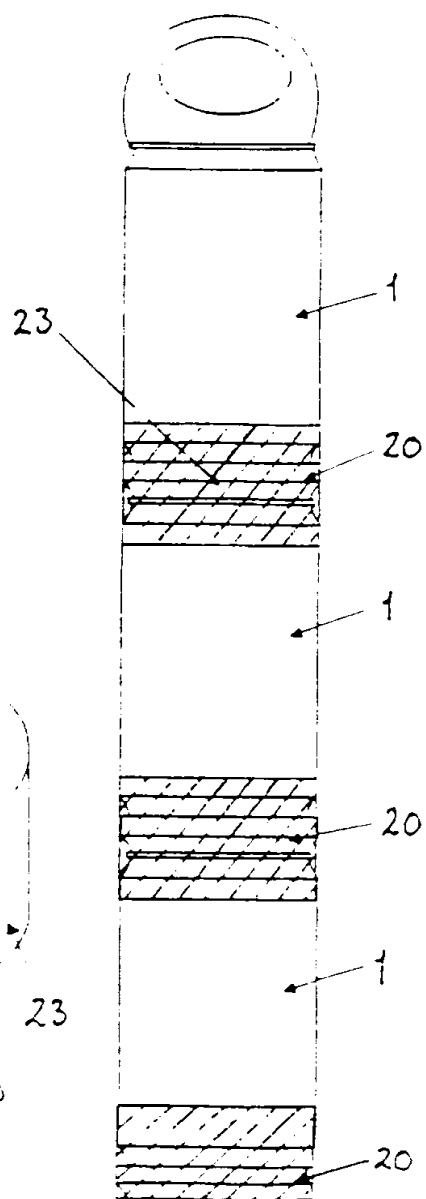
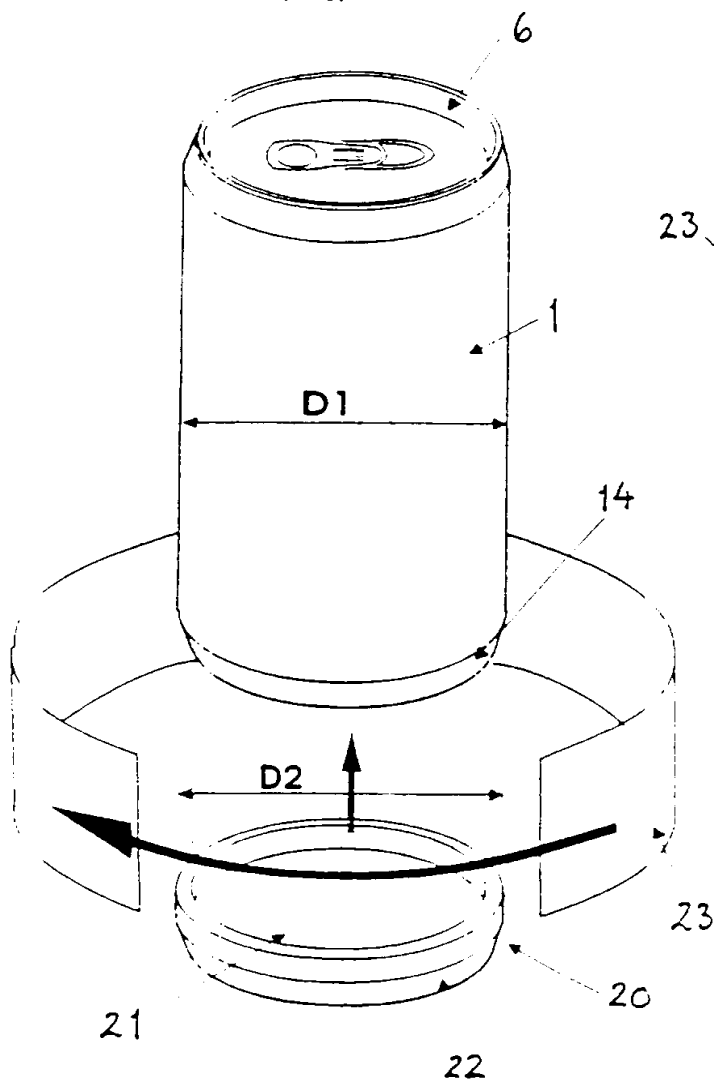


FIG. 15

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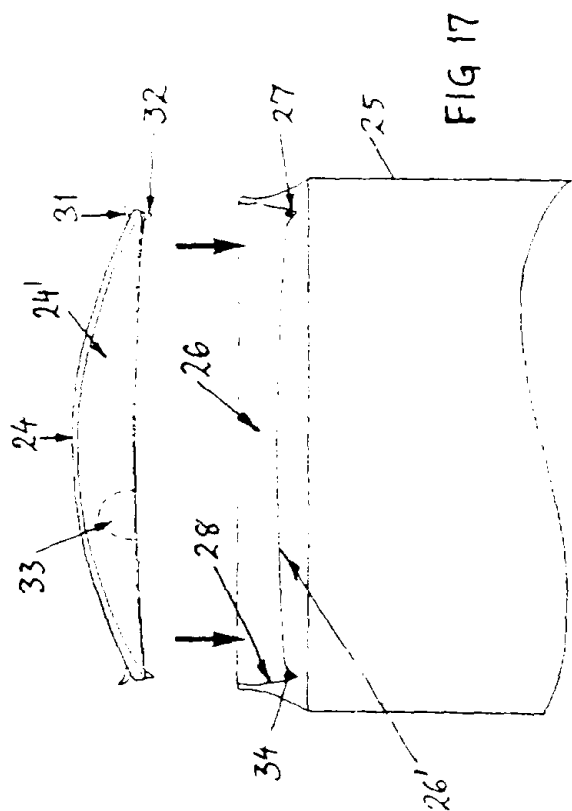


FIG. 17

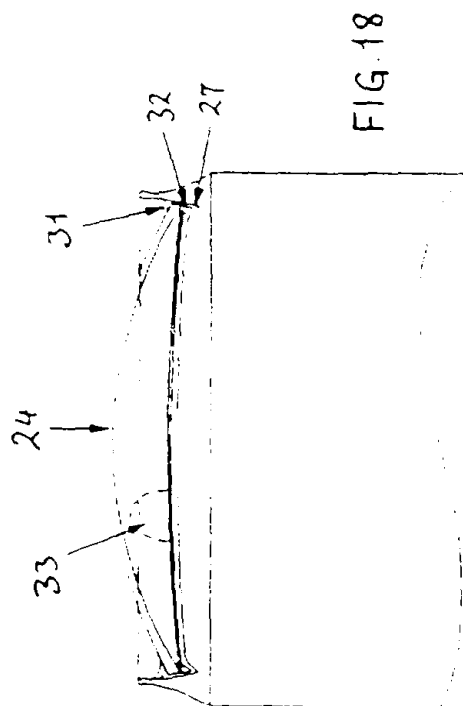


FIG. 18

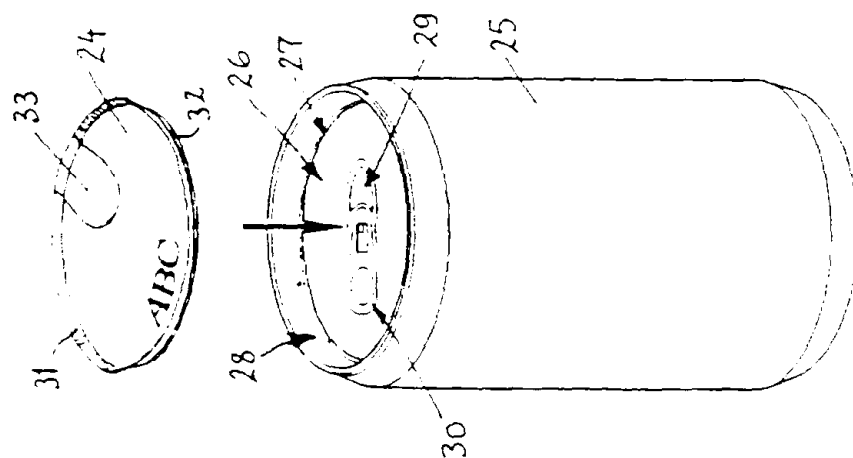


FIG. 16



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FIG. 19

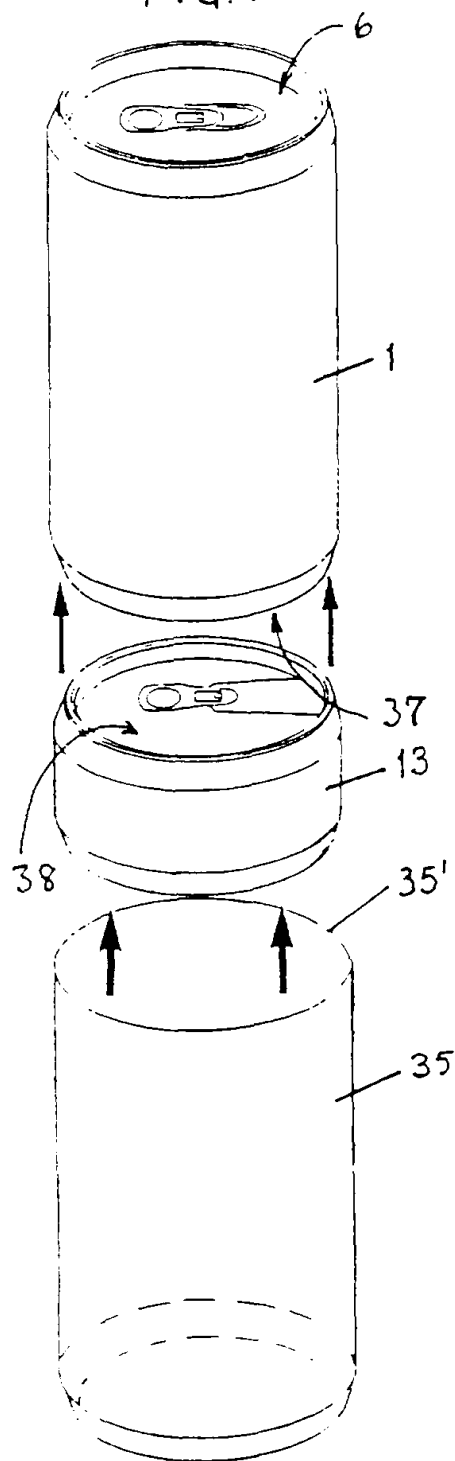


FIG. 20

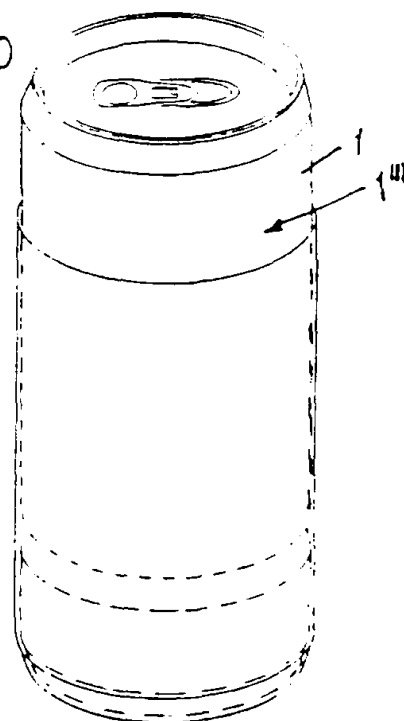
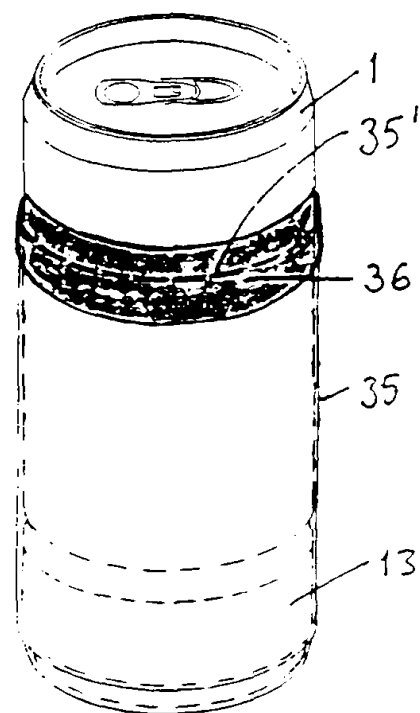


FIG. 21



## INTERNATIONAL SEARCH REPORT

International application No.

PCT/NO 96/00277

## A. CLASSIFICATION OF SUBJECT MATTER

IPC6: B65D 51/24, B65D 51/28, B65D 77/24  
According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: B65D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 3638787 A (PHILLIPS JR.), 1 February 1972 (01.02.72), figure 6, abstract --	2,7,8,9,14, 15
X	US 5339975 A (STONER), 23 August 1994 (23.08.94), figures 2,5,9,18-20, abstract --	2,6,9,14,15
X	NO 39104 C (HJALMAR GUSTAF NYSTRÖM), 16 June 1924 (16.06.24), page 1, line 1 - line 11, figure 1 --	2,8,9,14,15
A	US 2804201 A (S.F. CHAPPELL), 27 August 1957 (27.08.57), figures 5,6 --	1

☒ Further documents are listed in the continuation of Box C.☒ See patent family annex.

## \* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

17 March 1997

Date of mailing of the international search report

18 -03- 1997

Name and mailing address of the ISA:

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Telephone No. +46 8 782 25 00



# INTERNATIONAL SEARCH REPORT

International application No.

PCT/NO 96/00277

## Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Claim 1 concerning a cover with two tongues on its, backside, where the tongues are attached to the opening of a can.

Claims 2 and 6-15 concerning a cover in the form of a can that has an axial extension that is much smaller than the main can.

Claims 3-5 concerning a cover with a sealinglip and a bulge that corresponds to a recess in a can.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☒ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.  
☐ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

04/03/97

International application No

PCT/NO 96/00277

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
US-A-	3638787	01/02/72	NONE		
US-A-	5339975	23/08/94	AU-A-	9030291	11/06/92
			US-A-	5154295	13/10/92
			WO-A-	9208659	29/05/92
NO-C-	39104	16/06/24	NONE		
US-A-	2804201	27/08/57	NONE		
US-A-	5370262	06/12/94	JP-A-	7040984	10/02/95
US-A-	5273176	28/12/93	NONE		
US-A-	4917258	17/04/90	NONE		
US-A-	2120403	14/06/38	NONE		
WO-A1-	9604181	15/02/96	AU-A-	3183095	04/03/96
			GB-D-	9415782	00/00/00





(19) **BUNDESREPUBLIK  
DEUTSCHLAND**



**DEUTSCHES  
PATENTAMT**

(12) **Offenlegungsschrift**  
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(51) Int. Cl.<sup>6</sup>:  
**B 65 D 17/32**  
B 65 D 17/34

(21) Aktenzeichen: 197 04 148.5  
(22) Anmeldetag: 4. 2. 97  
(43) Offenlegungstag: 13. 8. 98

DE 197 04 148 A 1

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(72) Erfinder:  
gleich Anmelder  
(56) Entgegenhaltungen:  
DE 87 13 226 U1  
WO 95 28 328  
WO 89 11 421

**Die folgenden Angaben sind den vom Anmelder eingereichten Unterlagen entnommen**

Prüfungsantrag gem. § 44 PatG ist gestellt

(53) Dose mit Verschuß

(57) Die Erfindung betrifft eine Dose mit einem Verschuß von der Art, daß sie einen Zugaufsatz bzw. Aufreißring aufweist, welche Dose hygienisch ist, eine Verletzung des Benutzers und ein Verspritzen bzw. Versprühen des Inhalts verhindern und wiederverwendet werden kann. Der Verschuß wird an der einen Aufreißring aufweisenden Dose dergestalt angebracht, daß die Dosenöffnung überdeckt wird.

DE 197 04 148 A 1

Diese Erfindung bezieht sich auf Dosen bzw. Getränkedosen der Art, die mit einer Zuglasche bzw. Aufreißring versehen sind, insbesondere für Dosenbier und Dossensaft, und genauer auf eine Dose mit einer Deckelkappe bzw. einem Verschluss, die bzw. der einen beweglichen Öffnungs- bzw. Auslaßverschluss abdeckt bzw. verschließt.

**Fig. 11** zeigt eine mit einem Aufreißring versehene Getränkedose gemäß dem Stand der Technik, in die Bier oder andere Getränke abgefüllt wird. Wie abgebildet, umfaßt die Dose einen zylindrischen Dosenkörper **1** mit einem Boden. Ihr Deckel weist einen Aufreißring **3** auf, der durch Ziehen in die Richtung des Pfeiles gehoben werden kann. Wie in **Fig. 12** gezeigt wird durch Ziehen und Hochheben des Aufreißringes **3** zum Trinken des Inhaltes ein einreißbarer Verschluss **6a** bzw. eine Aufreißblase eingerissen und in die Dose eingebracht, so daß eine Öffnung **6** als eine Dosenöffnung gebildet wird.

Wie in **Fig. 11** gezeigt, kann eine Verunreinigung (a), wenn sie sich an einem Dosendeckelabschnitt in der Nähe der Dosenöffnung befindet, in die Dose gelangen, wenn die Dose geöffnet wird, oder sie kann in den Körper von der Person gelangen, die den Inhalt trinkt. Manchmal kann die Verunreinigung eine Krankheit herbeiführen.

Ein anderes Problem ist, daß die Kante der geöffneten Dosenöffnung scharf wie eine Rasierklinge ist und Finger verletzen kann. Insbesondere ist dies gefährlich für Kleinkinder und Kinder.

Zur Zeit werden die "PL", insbesondere Produkthaftungs-Bestimmungen, die die Produkthaftung betreffen, durchgesetzt, und die Hersteller müssen gewisse Maßnahmen vorsehen, um das oben genannte Problem zu lösen.

Wie in **Fig. 12** gezeigt, ist ein weiteres Problem, daß der Inhalt der Dose nach außen hin verspritzt werden kann, um Kleidungsstücke oder in der Nähe befindliche Dinge zu verunreinigen, wenn der Aufreißring **3** während des zu Fuß Gehens betätigt wird oder in einem rüttelnden Eisenbahnzug oder in einem geneigten Zustand der Dose oder in Abhängigkeit von der Art, wie eine Kraft zum Heben des Aufreißringes angewendet wird.

Bei einer anderen Art von Dose ist ein dünner Plastikdeckel praktisch an die Dosenoberseite angepaßt. Solch ein Deckel wird leicht während des Transportes oder der Handhabung der Dose gelöst bzw. abgenommen. Außerdem kann der Deckel nicht mehr länger richtig aufgesetzt werden, wenn er einmal abgenommen wurde.

Die Erfindung wurde im Hinblick auf die obengenannten Probleme gemacht und ihre Aufgabe ist es, eine Dose mit einem Verschluss bereitzustellen, die hygienisch ist, einen zufälligen Schaden unterbinden kann, ein Ausspritzen des Inhaltes verhindern kann und geeignet zur Wiederverwendung ist.

Ein Merkmal der Erfindung, um die oben genannte Aufgabe zu lösen, ist es, eine Dose mit einem Verschluss bereitzustellen, wobei die Dose einen Oberseiteckel mit einem Aufreißring aufweist, der Verschluss auf seiner Innenseite mit einem Kleber versehen ist und an der Dose dergestalt angebracht ist, daß er abgestreift bzw. abgehoben werden kann, und der Verschluss eine Dosenöffnung umwandelt, die durch Ziehen und Hochheben des Aufreißringes geöffnet werden kann.

Der Verschluss ist im wesentlichen halbkreisförmig oder kreisförmig und weist einen Zugabschnitt auf.

Der Verschluss ist eine antibakterielle Verschluss.

Der Kleber enthält ein antibakterielles Mittel.

Der Verschluss weist einen Falzabschnitt auf, der an die Dose geklebt werden soll, der von seiner bogenförmigen

Kante abhängt und sich hinunter zu einem Abschnitt erstreckt, um an den unteren Auslaß bzw. die Lippe anzugreifen.

**Fig. 1** ist eine perspektivische Teilansicht, die eine Ausführungsform der Erfindung zeigt.

**Fig. 2** ist eine perspektivische Ansicht, die einen Verschluss zeigt, die in der Ausführungsform der Erfindung verwendet wird.

**Fig. 3** ist eine Darstellung, welche die Vorgehensweise des Öffnens der Ausführungsform der erfindungsgemäßen Dose zeigt.

**Fig. 4** ist eine Ansicht, welche die Dose zeigt nachdem sie geöffnet worden ist, um bereit zum Trinken des Inhaltes zu sein.

**Fig. 5** ist eine Ansicht, die eine Vorgehensweise zum Anbringen des erfindungsgemäßen Verschlusses darstellt.

**Fig. 6** ist eine Ansicht, die eine Fortsetzung der Vorgehensweise zum Anbringen des Verschlusses darstellt.

**Fig. 7** ist eine Ansicht, die eine andere Vorgehensweise zum Anbringen der erfindungsgemäßen Verschlusses darstellt.

**Fig. 8** ist eine Ansicht, die ein abweichendes Beispiel des erfindungsgemäßen Verschlusses zeigt.

**Fig. 9** ist eine Ansicht, die eine abweichende Ausführungsform der Erfindung zeigt.

**Fig. 10** ist eine Ansicht, die eine weitere Ausführungsform der Erfindung zeigt.

**Fig. 11** ist eine Ansicht, die eine Dose mit einem Verschluss gemäß dem Stand der Technik zeigt; und

**Fig. 12** ist eine Ansicht, welche die Dose gemäß dem Stand der Technik in einem anderen Zustand zeigt.

**Fig. 1** ist eine perspektivische Teilansicht, die eine Ausführungsform der Erfindung zeigt. Bezugnehmend auf die Figur wird mit **1** eine gewöhnliche Dose, wie z. B. eine Aluminiumdose, bezeichnet. Die Dose **1** umfaßt einen zylindrischen Körper **2** mit einem Boden. Angepaßt an das Oberteil des Dosenkörpers **2** ist ein scheibenähnlicher Deckel **4** mit einem Aufreißring **3**, der eine wohlbekannte Struktur aufweist.

Mit **5** wird ein Verschluss bezeichnet, der aus einer dünnen Folie, einer Aluminiumfolie etc. hergestellt ist. Der Verschluss **5** ist im wesentlichen in seiner Form halbkreisförmig und weist einen Falzabschnitt **5a** auf, der sich nach unten gerichtet von seiner bogenförmigen Kante erstreckt. Der Falzabschnitt **5a** kann zu einem großen Teil den Deckel **4** überdecken. Obwohl nicht gezeigt, ist der Verschluss **5** zumindest teilweise kronen- bzw. kappenartig und überdeckt einen Rand- bzw. Umfangsabschnitt der Dose **1**, um von der unteren Lippe berührt zu werden. Diese Anordnung ist sehr hygienisch. Der Verschluss **5** weist einen Zugabschnitt **5b** auf, der im wesentlichen in der Mitte der bogenförmigen Kante gebildet ist. Der Verschluss **5** kann von der Dose **1** durch Klemmen des Zugabschnitts **5b** zwischen die Finger nach oben gezogen werden. Der Verschluss **5** weist ein Fenster **5c** auf, das im wesentlichen in dem Mittelabschnitt seines Oberteils gebildet und aus einem transparenten oder semitransparenten Material hergestellt ist. Das Fenster **5c** kann eine beliebige Form aufweisen und auch eine beliebige Größe, solange es ermöglicht, daß der Zustand der Öffnung **6** leicht von der Außenseite gesehen bzw. erkannt werden kann.

**Fig. 2** ist eine perspektivische Ansicht und zeigt den Verschluss **5**. Wie schematisch gezeigt, wird ein Klebstoff von einer Art, der relativ leicht aufgetragen und von der klebenden Oberfläche abgegrenzt und auch wieder verwendet werden kann, auf der Innenfläche des Falzabschnitts **5a** und auch auf den Innenflächen eines Aufhals- bzw. Vorderabschnittes **5d** des Zugabschnitts **5b** und eines Stirnkantenabschnittes **5b'**.



und, wie in Fig. 1 gezeigt, ist der Verschluss 5 vorgesehen, um im wesentlichen eine Hälfte des Deckels 4 zu überdecken, der den Öffnungsverschluss bzw. die Aufreißblase 6 aufweist, die von dem Aufreißring 3 geöffnet werden kann. Es ist möglich, den Klebstoff auf der gesamten Innenfläche des Verschlusses zu beschichten.

Der Verschluss 5 kann aus irgendeinem Material hergestellt werden. Das Material muß jedoch haltbar bzw. widerstandsfähig im Hinblick auf Wasser sein und schwierig zu zerbrechen bzw. einzudrücken. Geeigneterweise ist das Material antibakteriell behandelt und weist antibakterielle Eigenschaften auf, weil der Inhalt der Dose durch den Mund in den Körper aufgenommen wird. Die antibakterielle Behandlung kann durch Beschichtung oder durch beschichtendes Einführen eines antibakteriellen Mittels vorgenommen werden, das wirksam gegen Kolibakterien ("colon-Bazillen"), MRSA (Methicillin-resistente Staphylococcus aureus), Salmonellen etc. ist, auf oder in mindestens einem Abschnitt der Innenfläche des Verschlusses 5 in der Nähe der Dosenöffnung. Natürlich ist es möglich, die gesamte Innenfläche des Verschlusses der antibakteriellen Behandlung zu unterziehen. Wo der Verschluss 5 aus einem lichtundurchlässigen Material hergestellt ist, ist das Fenster 5c aus einem transparenten oder semitransparenten Material, wie oben bemerkt, zur Beobachtung des Abschnittes 6 vorgesehen, welcher der Dosenöffnung entspricht. Wo jedoch der Verschluss 5 selbst aus einer dünnen transparenten oder semitransparenten Folie hergestellt ist, ist es nicht notwendig, ein Fenster 5c vorzugeben. Anstelle der Verwendung eines antibakteriellen Verschlusses kann das antibakterielle Mittel seinerseits in dem Klebstoff eingebracht werden, um das Eindringen von Bakterien von der Außenseite zu verhindern.

Fig. 3 zeigt die Art, wie die Dose mit dem Verschluss geöffnet wird. Zuerst wird der Aufreißring 3 gehoben, wie bei (1) gezeigt. In einer zu diesem Vorgang verknüpften Beziehung wird der Abschnitt 6 in die Dose eingebracht bzw. eingeführt, so daß dadurch die Dosenöffnung geöffnet wird. Dieser Zustand kann leicht durch das Fenster 5c des Verschlusses 5, der aus einem lichtundurchlässigen Material hergestellt ist, überprüft werden. In diesem Stadium ist die Dosenöffnung von dem Verschluss 5 überdeckt, um ein Anfügen von Verunreinigung daran zu verhindern. Daraufhin wird der Zugabschnitt 5b durch Klemmen zwischen die Finger gehoben. Wie in Fig. 4 gezeigt, wird die Dosenöffnung als ein Ergebnis geöffnet, so daß sie bereitsteht zum Trinken des Inhaltes.

Wenn der Inhalt vollständig ausgetrunken worden ist, kann der Verschluss 5 wieder nach unten gebracht werden, um wieder die Dosenöffnung zu überdecken. Indem dies so gemacht wird, ist es nicht länger möglich, daß Finger an der scharfen Kante der Dosenöffnung verletzt werden könnten.

Wenn es erwünscht ist, den übriggebliebenen Teil des Inhaltes aufzubewahren, nachdem ein Teil davon getrunken worden ist, kann die Dose hygienisch in einem Kühlschrank oder einem ähnlichen Gerät aufbewahrt werden, nachdem die Dosenöffnung 6 mit dem Verschluss 5 geschlossen worden und der Verschluss gesichert an der Dose angebracht worden ist, indem z. B. Gebrauch von einer Restklebekraft des Klebstoffs gemacht wird. Der Verschluss 5 kann ebenfalls als ein Anzeigeabschnitt verwendet werden, indem eine notwendige Beschreibung oder etwas ähnliches darauf geschrieben wird.

Fig. 5 und 6 stellen ein Beispiel der Vorgehensweise zum Anbringen des Verschlusses 5 dar. Wie in Fig. 5 gezeigt, wird der Verschluss 5 zunächst unter Verwendung eines Klebstoffes dergestalt angelegt, daß er im wesentlichen eine Hälfte des Deckels 4 überdeckt. Dann wird der Aufreißring 6, wie in Fig. 6 gezeigt, an dem Oberteil der Dose ange-

bracht, um die Dose zu verschließen.

Fig. 7 zeigt ein anderes Beispiel des Anbringens dem Verschluss 5. In diesem Fall wird der Verschluss 5 aufgesetzt, nachdem der Deckel 4 mit dem Aufreißring an der Dose angebracht worden ist. In diesem Beispiel ist die Handhabe bzw. der Hebel des Aufreißringes 3 ebenso von dem Verschluss 5 überdeckt. Jedoch kann der Verschluss 5 auch von der in Fig. 1 gezeigten Form sein.

Fig. 8 zeigt ein abweichendes Beispiel des Verschlusses 5. Dieses Beispiel des Verschlusses 5 weist keinen Falzabschnitt 5a auf, wie er in Fig. 2 gezeigt wurde. Wie in Fig. 9 gezeigt, wird in diesem Fall ein innerer Kanten- bzw. Randabschnitt auf den Deckel 4 aufgesetzt. In Fig. 8 ist ein Abschnitt schattiert dargestellt, auf dem der Klebstoff vorgesehen ist. Während der oben beschriebene Verschluss 5 im wesentlichen halbkreisförmig in seiner Grundform gewählt ist, kann der Verschluss ebenfalls mehr als eine Hälfte des Deckels überdecken oder eine abweichende Form aufweisen.

Fig. 10 zeigt ein Beispiel, in dem ein Verschluss 5a auf eine Dose 1A aufgesetzt wird, die verarbeitete Nahrung bzw. ein Fertiggericht enthält, z. B. Fleisch oder Fisch oder Meeresfrüchte. Die Dose 1A dieses Typs wird gewöhnlicherweise dadurch geöffnet, daß der halbkreisförmige obere Deckel 4A im wesentlichen vollständig entfernt wird. Dem entsprechend ist der Verschluss 5a im wesentlichen kreisförmig.

Wie im Anspruch 1 dargestellt, wird mit dem Bereitstellen des Verschlusses 5 (oder 5A), der wie oben erwähnt abgestreift bzw. abgehoben werden muß, die Dosenöffnung sauber gehalten werden, und diese kann durch Ziehen und Hochheben des Aufreißringes 3 geöffnet werden, ohne daß der Inhalt nach außen hin verspritzt wird und die Umgebung verunreinigt wird. Nach Trinken eines gewissen Teiles des Inhaltes kann die Dose durch Überdecken bzw. Abdichten der Dosenöffnung mit dem Verschluss 5 (oder 5A) gelagert werden. Indem die Dosenöffnung wieder mit dem Verschluss 5 (oder 5A) und der Verschluss wieder an der Dose mit Hilfe des Klebstoffs angebracht wird, nachdem der Inhalt vollständig ausgetrunken wurde, wird die Möglichkeit einer zufälligen Verletzung ausgeschlossen.

Wie im Patentanspruch 2 dargelegt, kann der Zugabschnitt 5b des Verschlusses 5 (oder 5A) einfach geklemmt werden und ermöglicht, daß der Verschluss 5 (oder 5A) leicht abgestreift bzw. abgehoben werden kann.

Wie im Patentanspruch 3 dargelegt, kann der antibakterielle Verschluss ein Eindringen oder ein Ausbreiten von Bakterien unterdrücken, und sie ist sehr hygienisch.

Wie im Patentanspruch 4 dargelegt, kann der Klebstoff, der ein antibakterielles Mittel enthält, ein Eindringen und Ausbreiten von Bakterien unterdrücken.

Wie im Patentanspruch 5 dargelegt, ist es nahezu vollständig möglich, ein Eindringen von Verunreinigung in die Dose zu verhindern, indem man den Verschluss 5 (oder 5A) mit dem Falzabschnitt 5a versieht, der an den zylindrischen Körper der Dose 1 (oder 1A) geklebt bzw. befestigt ist.

Die Erfindung betrifft eine Dose mit einem Verschluss von der Art, daß sie einen Zugaufsatz bzw. Aufreißring aufweist, welche Dose hygienisch ist, eine Verletzung des Benutzers und ein Verspritzen bzw. Versprühen des Inhaltes verhindern und wiederverwendet werden kann. Der Verschluss (5, 5A) wird an der einen Aufreißring aufweisenden Dose (1, 1A) dergestalt angebracht, daß die Dosenöffnung überdeckt wird.

#### Patentansprüche

1. Dose mit Verschluss, wobei die Dose einen oberen Deckel mit einem Aufreißglied bzw. einer Zuglasche

aufweist, der Verschuß auf seiner Innenseite mit einem Kleber versehen ist und dergestalt an der Dose angebracht ist, daß er sich oberhalb von dieser befindet, um abgezogen bzw. abgehoben zu werden, wobei der Verschuß eine Öffnung bzw. einen Auslaßverschuß überdeckt, die bzw. der durch Ziehen und Hochheben des Aufreißgliedes bzw. der Zuglasche geöffnet werden kann.

2. Dose mit Verschuß nach Anspruch 1, bei welcher der Verschuß im wesentlichen halbkreisförmig oder kreisförmig ist und einen Zugabschnitt aufweist.

3. Dose mit Verschuß nach Anspruch 1 oder 2, bei welcher der Verschuß ein antibakterieller Verschuß ist.

4. Dose mit einer Verschuß nach einem der vorhergehenden Ansprüche, bei welcher der Klebstoff ein antibakterielles Mittel enthält.

5. Dose mit Verschuß, bei welcher der Verschuß einen Falzabschnitt aufweist, der an die Dose geklebt bzw. mit dieser verbunden wird und der von ihrer bogenförmigen Kante abhängt und sich nach unten zu einem Abschnitt erstreckt, um an die untere Lippe bzw. den Ausguß anzugrenzen.

Hierzu 4 Seitenm Zeichnungen

25

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FIG.1

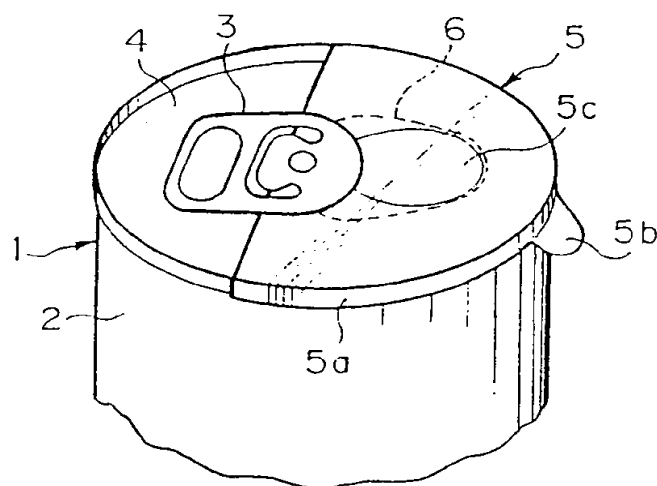


FIG.2

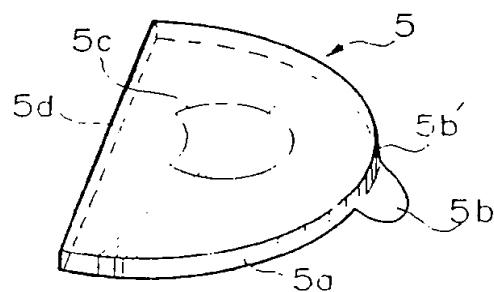


FIG.3

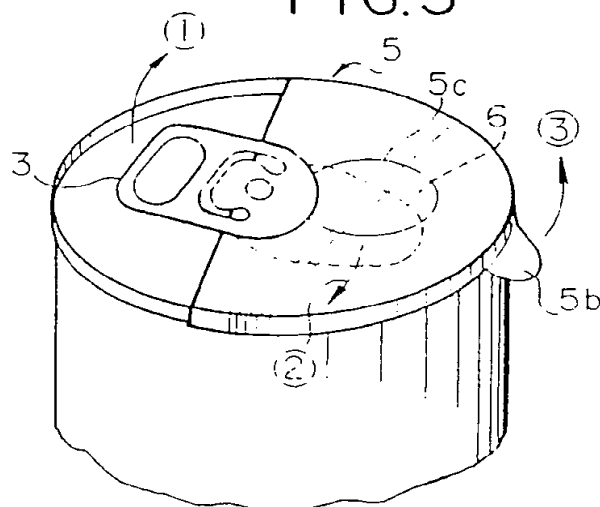


FIG.4

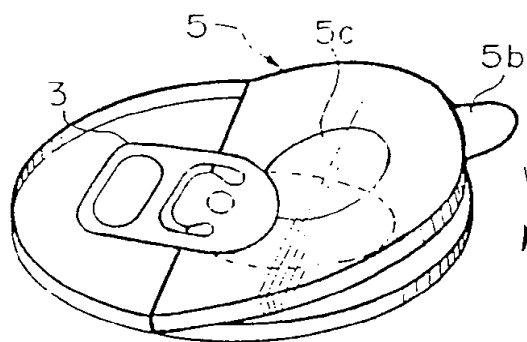


FIG.5

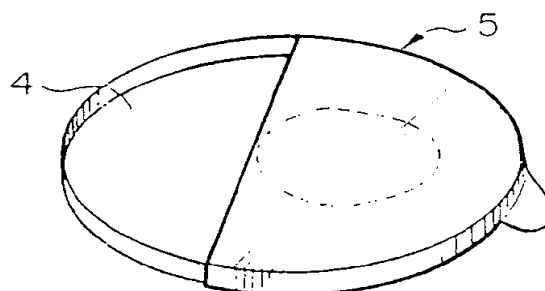


FIG.6

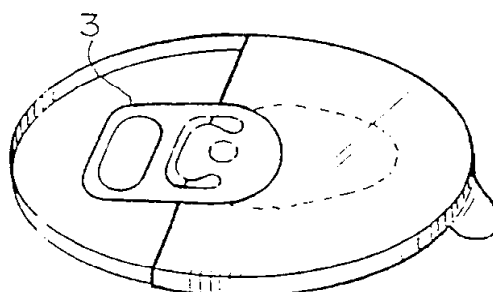


FIG. 7

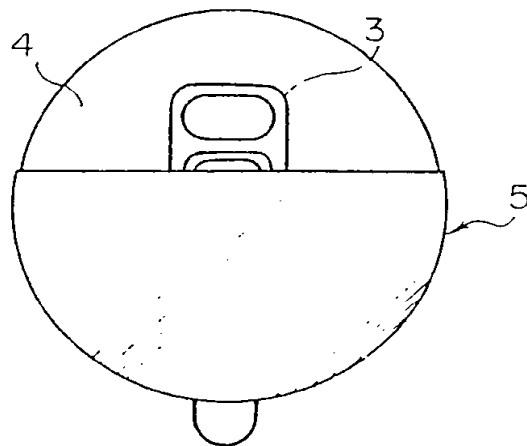


FIG. 8

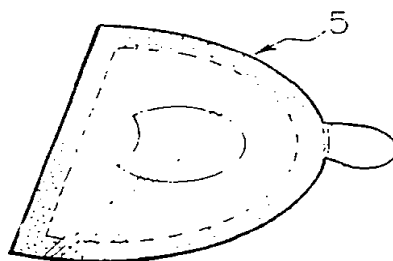


FIG.9

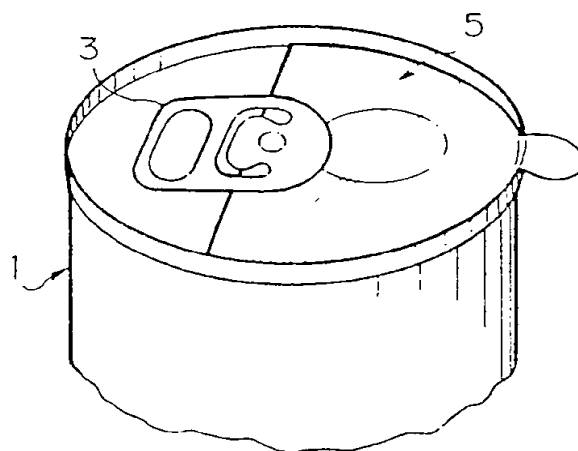


FIG.10

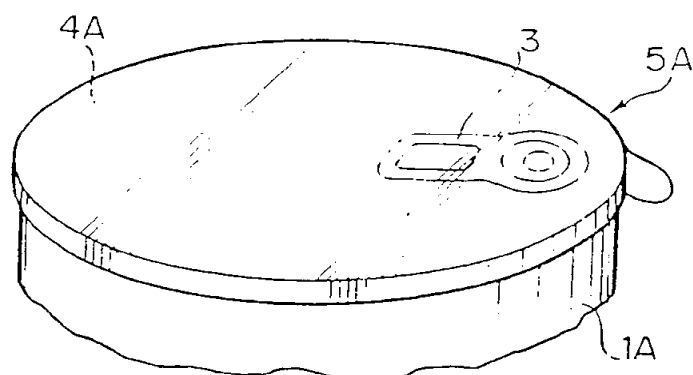


FIG.11

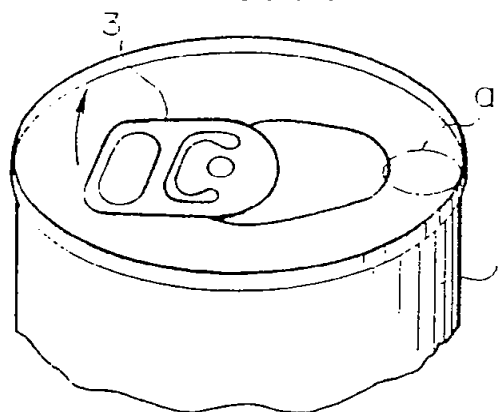
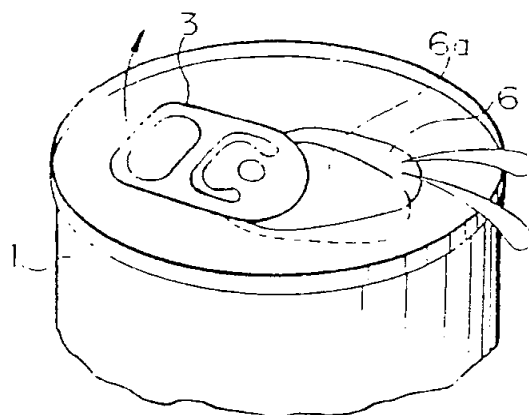


FIG.12



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(12) **DEMANDE DE BREVET D'INVENTION**

A1

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(71) Demandeur(s) : *Paul GRISON et Jean Pierre GRISON.* —  
FR.

(72) Inventeur(s) : Paul Grison ; Jean Pierre Grison.

(73) Titulaire(s) :

(74) Mandataire(s) : Paul Grison.

(54) Dispositif d'hygiène par adhérence pour boîtes métalliques de boissons.

(57) Dispositif pour conserver propre sur une boîte de bois-  
sons l'endroit où l'on pose les lèvres pour boire.

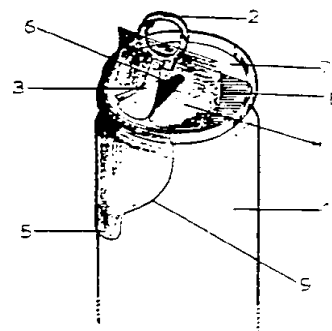
L'invention concerne un dispositif permettant de conserver  
hygiéniquement propre, malgré les diverses manipulations mé-  
caniques ou manuelles qu'elle a subi, la partie d'une boîte où  
l'on pose les lèvres.

Il est constitué d'une protection adhérente souple 4, fixée  
sur la partie supérieure 7 et sur le côté de la boîte 1. Solidaire  
de l'anneau de traction 2 et de la languette 3 de fermeture, à  
l'ouverture par le consommateur, ladite protection s'enlève,  
découvrant la partie conservée hygiéniquement propre, 8 grâce  
à elle. Elle peut être réutilisée pour refermer l'orifice 6 de la  
boîte 1.

A cet effet, l'arrondi 9 de la protection 4 a le même  
diamètre que la boîte 1 et une languette 5 en facilite l'utilisa-  
tion.

La protection 4 retenant par adhérence l'anneau 2 et la  
languette 3 évite ainsi qu'ils ne soient jetés au sol.

Le dispositif selon l'invention est particulièrement destiné  
aux boîtes de boissons.



FR 2 644 431 - A1

D

DISPOSITIF D'HYGIENE POUR BOITES METALLIQUES DE BOISSONS

1

La présente invention concerne un dispositif qui par adhérence sur la partie supérieure d'une boîte métallique contenant une boisson, permet de conserver hygiéniquement propre l'endroit où le consommateur pose ses lèvres pour boire.

5 Il existe sur le marché depuis des années, des boîtes métalliques de dimension standard, qui servent d'emballage pour des boissons gazeuses, sucrées ou alcoolisées.

10 L'ouverture ou décapsulage de ces emballages, se fait à l'aide d'un anneau de traction solidaire d'une languette métallique que l'on tire, permettant ainsi de pratiquer un orifice de forme triangulairement allongée.

A la fabrication de ces emballages, pendant le remplissage et jusqu'à la phase finale, les conditions d'hygiène sont draconiennes, et tiennent compte de critères de propreté innérants à toutes manipulations alimentaires.

15 Le problème d'hygiène commence dès la sortie des chaînes de mise en boîte. Il se produit alors un ensemble de manipulations qui sont, les conditionnements par quantités, leurs chargements sur véhicule, leurs transports, leurs livraisons et leurs distributions aux clients.

En explicitant ces différentes opérations, on prend conscience d'un problème d'hygiène extrêmement important.

20 En effet, les chargements, les transports et les livraisons sont effectués par moyens mécaniques ou motorisés polluant, dont les boîtes vont recueillir une certaine partie de pollution, en plus des poussières et agents salissants divers.

De plus, dans la dernière phase des opérations, se rajoute un élément qui est la manipulation manuelle du revendeur.

25 Celui-ci touchera physiquement chaque emballage, soit pour recharger les distributeurs automatiques, soit pour servir le client, soit pour la vente directe au public sur les plages ou lors de manifestations sportives.

30 Mécidalement parlant, cette manipulation manuelle directe n'est absolument pas hygiénique, puisqu'il peut y avoir transfert sur les boîtes à travers la transpiration, de microbes, de virus, ou de micoses épidermiques.



L'ensemble des points négatifs touchant à l'hygiène directe, relevés plus haut est très important, car après ouverture des boîtes par le consommateur, l'absorption de la boisson se fait en collant directement ses lèvres contre le récipient.

5 Il est aisé de comprendre qu'après l'ensemble des phases transports et manutentions subis par cet emballage, l'endroit où se posent les lèvres du consommateur ne peut être propre. Cela peut même être un facteur à haut risque compte tenu des manipulations humaines directes.

10 Le dispositif selon l'invention permet de remédier à ces inconvénients importants. Dans leur mode actuel, le dessus de ces boîtes comporte un système à traction composé d'une languette métallique solidaire d'un anneau que l'on tire, pour effectuer l'ouverture.

15 Le consommateur soulève cet anneau, puis tire sur la languette qui se détachera suivant une délimitation précise, provoquant l'ouverture de la boîte et l'accès à la boisson.

20 Cette boisson sera ensuite consommée en collant les lèvres contre le rebord de la boîte et en inclinant celle-ci. Pour éviter le contact direct des lèvres sur le rebord de la boîte qui n'est pas propre, compte tenu des manipulations antérieures, une protection adhérente souple qui pourrait être un capuchonnage de toute la partie supérieure de la boîte, est mise en place à un moment déterminé en usine, quand les conditions d'hygiène sont parfaites, et que la boîte est encore dans une zone de propreté absolue.

25 Selon une forme de réalisation préférentielle, cette protection adhérente souple a pour objet la préservation hygiénique parfaite d'un endroit déterminé sur la boîte, à l'endroit où se posent les lèvres du consommateur.

Cette protection adhérente souple va donc recouvrir la partie supérieure de la boîte, et le côté de celle-ci, correspondant à l'orifice d'ouverture puis- que c'est l'endroit où le consommateur pose ses lèvres, après ouverture de ladite boîte.

30 En recouvrant la partie supérieure de la boîte, cette protection adhérente souple est solidaire de la totalité de la languette et d'une partie de l'anneau.

Pendant l'opération d'ouverture de la boîte, le consommateur soulève, puis tire l'anneau et la languette, provoquant en même temps le décollement de la protection adhérente souple.

Une fois la languette détachée complètement de la boîte, à la fin de l'ouverture, elle reste toujours solidaire de la protection qui adhère encore à la partie supérieure, et sur le côté de la boîte.

5 En tenant toujours l'anneau, solidaire de la languette métallique, le consommateur continue de décoller la protection adhérente souple, jusqu'à la décoller entièrement. Il met ainsi à jour la partie de la boîte qui a toujours été protégée hygiéniquement durant tous les transports et les manipulations même dans des conditions douteuses.

10 Le consommateur, adulte ou enfant, aura ainsi la certitude absolue que l'endroit où il posera ses lèvres, sera hygiéniquement propre, puisque en effectuant cette opération d'ouverture de la boîte, il décollera en même temps la protection adhérente souple.

Cette invention a une autre application. Il arrive très souvent que la boisson contenue dans la boîte ne soit pas consommée en une seule fois après ouverture. 15 C'est souvent le cas pour un enfant. Cette éventualité peut se produire également lors d'un déplacement, pendant la conduite d'un véhicule, et dans toutes situations où la boisson risque de se renverser entre deux absorptions.

D'où l'utilité de pouvoir refermer hermétiquement l'orifice de la boîte.

20 A cet effet, la retombée inférieure de la protection adhérente souple a le même diamètre que l'intérieur de la partie supérieure ronde de la boîte. Les possibilités d'adhérence de la protection permettent de la recoller au-dessus de la boîte, refermant ainsi l'orifice, et autorisant plusieurs manoeuvres d'ouverture et de fermeture.

25 Ces manoeuvres seront facilitées par une petite languette de manipulation, située sur la partie inférieure de la protection adhérente souple, qui une fois repositionnée sur la partie supérieure de la boîte, dépassera à l'extérieur de la collerette de ladite boîte.

De plus, cette application selon l'invention apporte un élément de sécurité important. Chaque année, un certain nombre de personnes est piqué par des 30 HYMENOPTERES, attirés par le sucre contenu dans les boissons, et qui s'introduisent dans la boîte restée ouverte, piquant le consommateur qui boit le liquide.

En refermant l'orifice à l'aide de la protection adhérente souple, on évite ce type d'accident qui peut avoir des conséquences dramatiques, puisque les piqûres se situent dans la région buccale.

35 Cette application selon l'invention apporte également un autre avantage d'ordre écologique.

Il n'est pas rare de constater qu'en certains endroits, le sol est jonché de capsules d'ouverture de ces boîtes (anneaux solidaires de leurs languettes) alors que les boîtes, elles, ont été jetées dans les poubelles. Cela représente une pollution visuelle en même temps qu'un risque de blessure par coupure, sur les plages par exemple.

L'invention permet d'éviter ces nuisances, puisqu'après ouverture de la boîte, l'anneau et la languette restent solidaires de la protection adhérente. Cette protection étant réutilisée pour reboucher l'orifice de la boîte, l'anneau et la languette restent toujours solidaires de celle-ci. L'ensemble sera jeté dans une poubelle, évitant ainsi la nuisance évoquée.

La figure 1. représente de 3/4 le dispositif selon l'invention.

La figure 2. représente le début de la traction exercée sur l'anneau, et le décollement de la protection adhérente souple.

La figure 3. représente l'ouverture presque complète de l'orifice ainsi que le décollement de la protection.

La figure 4. représente vue de 3.4 arrière le décollement complet de la protection de la partie supérieure, et l'anneau et la languette collées à cette protection.

La figure 5. représente vue de profil, la protection, l'anneau et la languette en phase de décollement finale latérale.

La figure 6. représente la protection adhérente souple repositionnée sur la partie supérieure pour refermer l'orifice sur la boîte.

La figure 7. représente la protection adhérente souple en position d'ouverture par rapport à l'orifice.

La figure 1. représente une boîte standard (1) dont la partie supérieure (7) comporte le système de fermeture standard composé d'un anneau de traction (2) solidaire de la languette de fermeture (3).

La protection adhérente souple (4) est positionnée sur la partie supérieure (7) de la boîte et sur le côté correspondant au sens de l'ouverture.

Une petite languette (5) termine la protection adhérente souple (4).

Le diamètre de l'arrondi inférieur (9) de la protection adhérente souple est le même que le diamètre intérieur de la partie supérieure (7) de la boîte.

Ceci représente l'ensemble des dispositifs à l'achat de la boîte par le consommateur.

La figure 2. représente l'anneau de traction (2) en début de phase d'ouverture ainsi que le début de décollement de la protection adhérente souple (4) solidaire dudit anneau (2).

La languette métallique (3) est encore hermétiquement fermée. La partie hygiéniquement propre (8) commence à apparaître.

La figure 3. représente la suite de l'opération d'ouverture.

La traction sur l'anneau (2) provoque l'ouverture de l'orifice (6) de la  
5 boîte (1). Il entraîne le soulèvement de la languette métallique (3) et de la protection adhérente souple (4), ces trois éléments étant solidaires entre eux.

La partie hygiéniquement propre (8) continue d'apparaître.

La figure 4. représente vue d'arrière la protection adhérente souple (4) complètement décollée de la partie supérieure (7) de la boîte (1) et sur laquelle  
10 adhèrent l'anneau (2) et la languette (3).

La partie hygiéniquement propre (8) est complètement libérée sur la partie supérieure (7).

La figure 5. représente la fin du décollement de la protection adhérente souple (4). L'anneau (2) et la languette (3) adhèrent toujours sur la protection  
15 (4).

La fin de cette opération découvre complètement la partie (8), restée hygiéniquement propre grâce à la protection (4).

La figure 6. représente la protection adhérente souple (4) réutilisée comme moyen de fermeture de l'orifice (6). La languette (5) en saillie par rapport à  
20 la partie supérieure (7) de la boîte, facilite les manoeuvres d'ouverture et de fermeture de l'orifice (6) par ladite protection (4).

L'anneau (2) et la languette (3) en restant solidaires de la protection (4) évitent d'être jetés au sol.

La figure 7. représente la protection adhérente souple (4) en position  
25 d'ouverture, par rapport à l'orifice (6). Le reste de la protection (4) reste solidaire de la partie supérieure (7).

REVENDICATIONS

- 1) Dispositif d'une protection adhérente souple (4) de dimension non limitative destiné aux boîtes métalliques de boissons, caractérisé en ce que son adhérence lui permet de se fixer sur la partie supérieure et le côté desdites boîtes.
- 5        2) Dispositif selon la revendication 1), caractérisé en ce que la protection adhérente souple (4) recouvre une partie de l'anneau de traction (2) et entièrement la languette (3) pour descendre sur le côté de la boîte correspondant au sens de l'ouverture.
- 10       3) Dispositif selon l'une quelconque des revendications précédentes, caractérisé par le fait qu'en soulevant l'anneau (2) de traction, la protection adhérente souple (4) se soulève également.
- 15       4) Dispositif selon l'une quelconque des revendications précédentes caractérisé par le fait qu'en ouvrant complètement l'orifice de la boîte (1) par l'intermédiaire de l'anneau (2) et de la languette (3), la protection adhérente souple (4) se décolle entièrement de la partie supérieure (7), puisque solidaire de l'anneau et de la languette.
- 20       5) Dispositif selon l'une quelconque des revendications précédentes, caractérisé en ce qu'en continuant à exercer une traction sur l'anneau (2), la protection adhérente souple (4) se décolle totalement du côté de la boîte.
- 25       6) Dispositif selon l'une quelconque des revendications précédentes caractérisé en ce que cette protection adhérente souple (4) constitue la préservation hygiéniquement parfaite de la partie (8) où se posent les lèvres du consommateur.
- 30       7) Dispositif selon l'une quelconque des revendications précédentes, caractérisé en ce qu'après son décollement complet de la boîte, suite à l'ouverture, la protection adhérente souple (4) est réutilisée, grâce à sa forme arrondie (9) et à ses qualités auto-collantes, pour être repositionnée sur la partie supérieure (7) et obturer l'orifice (6) de ladite boîte. La petite languette (5) servant à faciliter les manoeuvres d'ouvertures.
- 35       8) Dispositif selon l'une quelconque des revendications précédentes, caractérisé en ce que les qualités d'adhérence de la protection souple (4) permettent de conserver solidairement à ladite protection, l'anneau (2) et la languette (3) après l'ouverture, évitant par ce fait, les problèmes de pollution que constitue le rejet au sol de ces deux éléments.

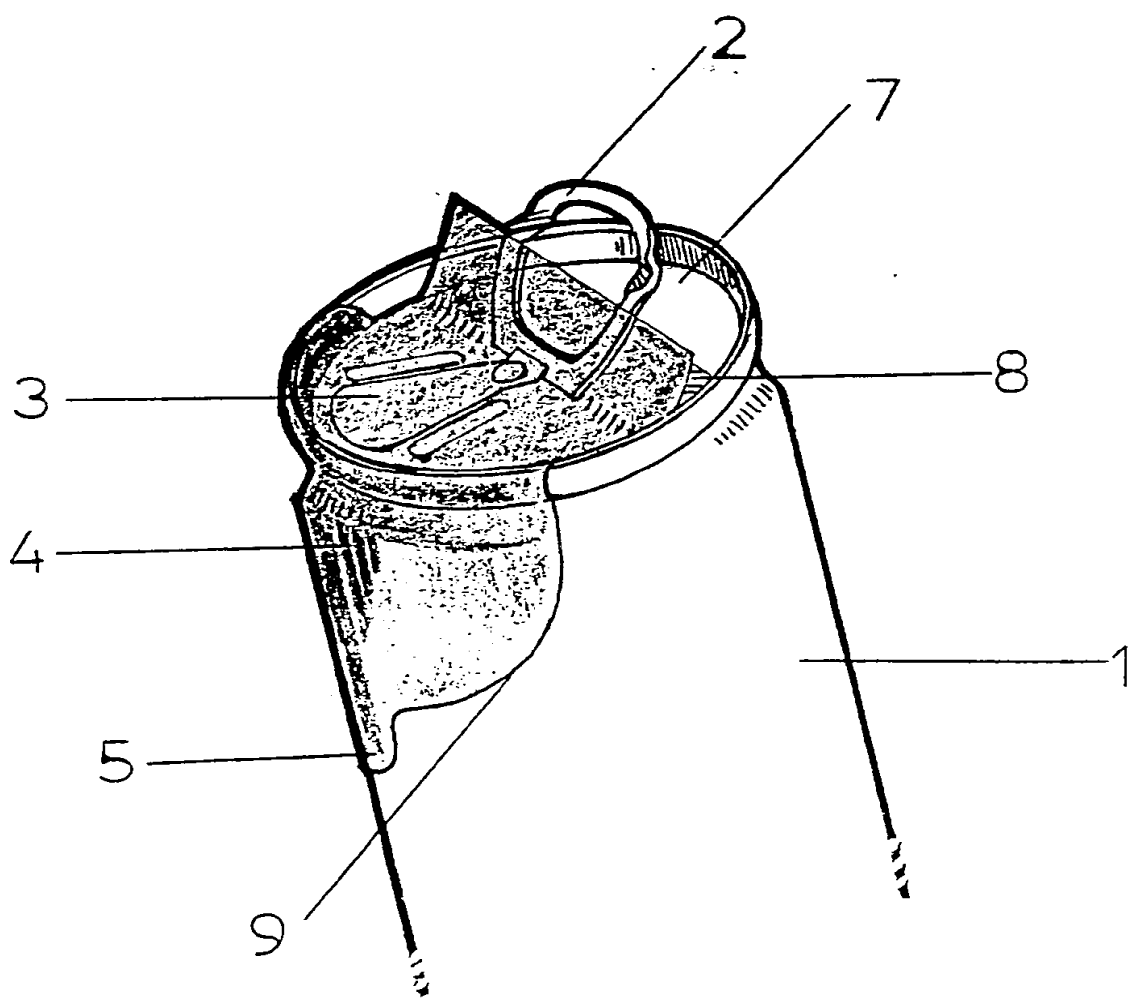


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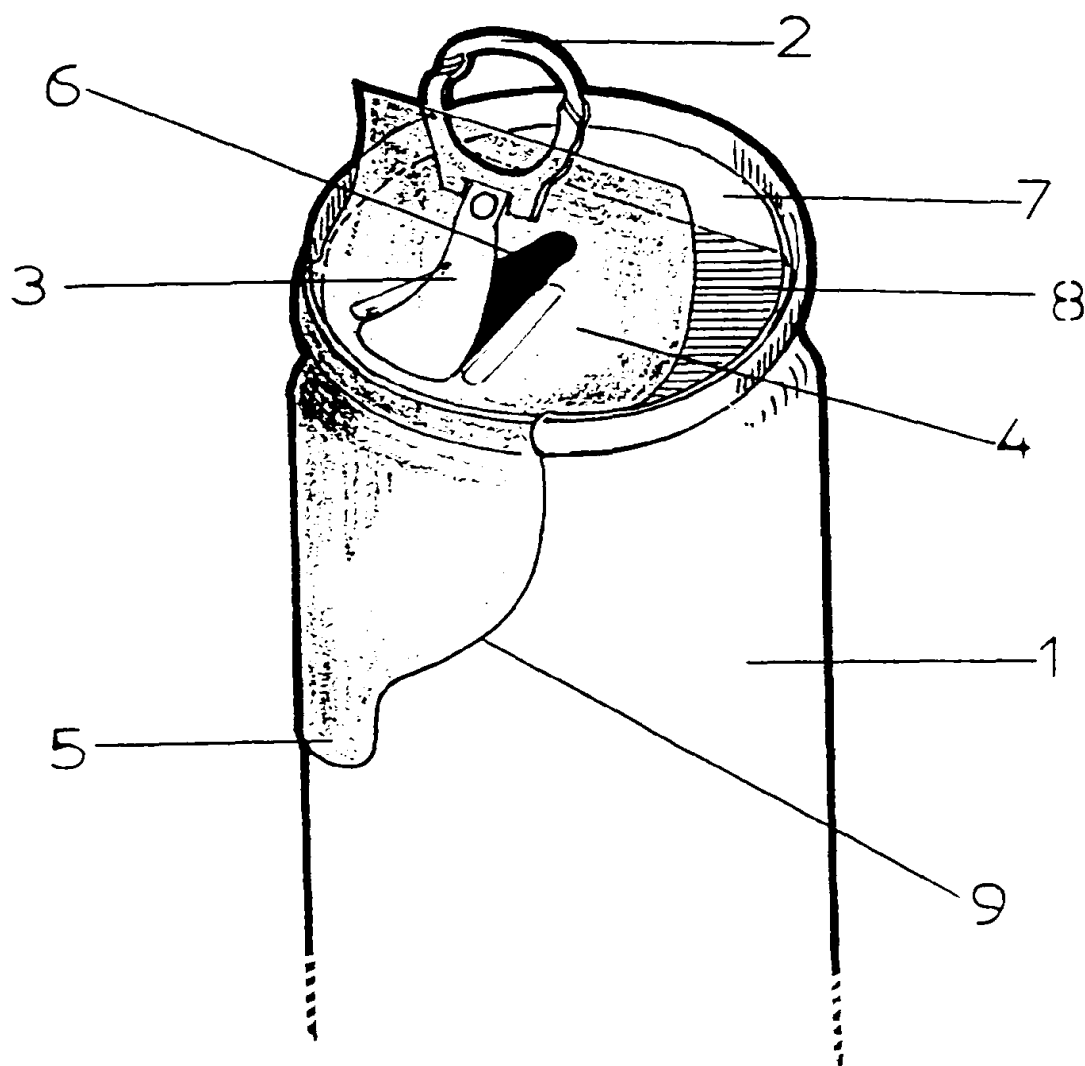
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FIGURE 2



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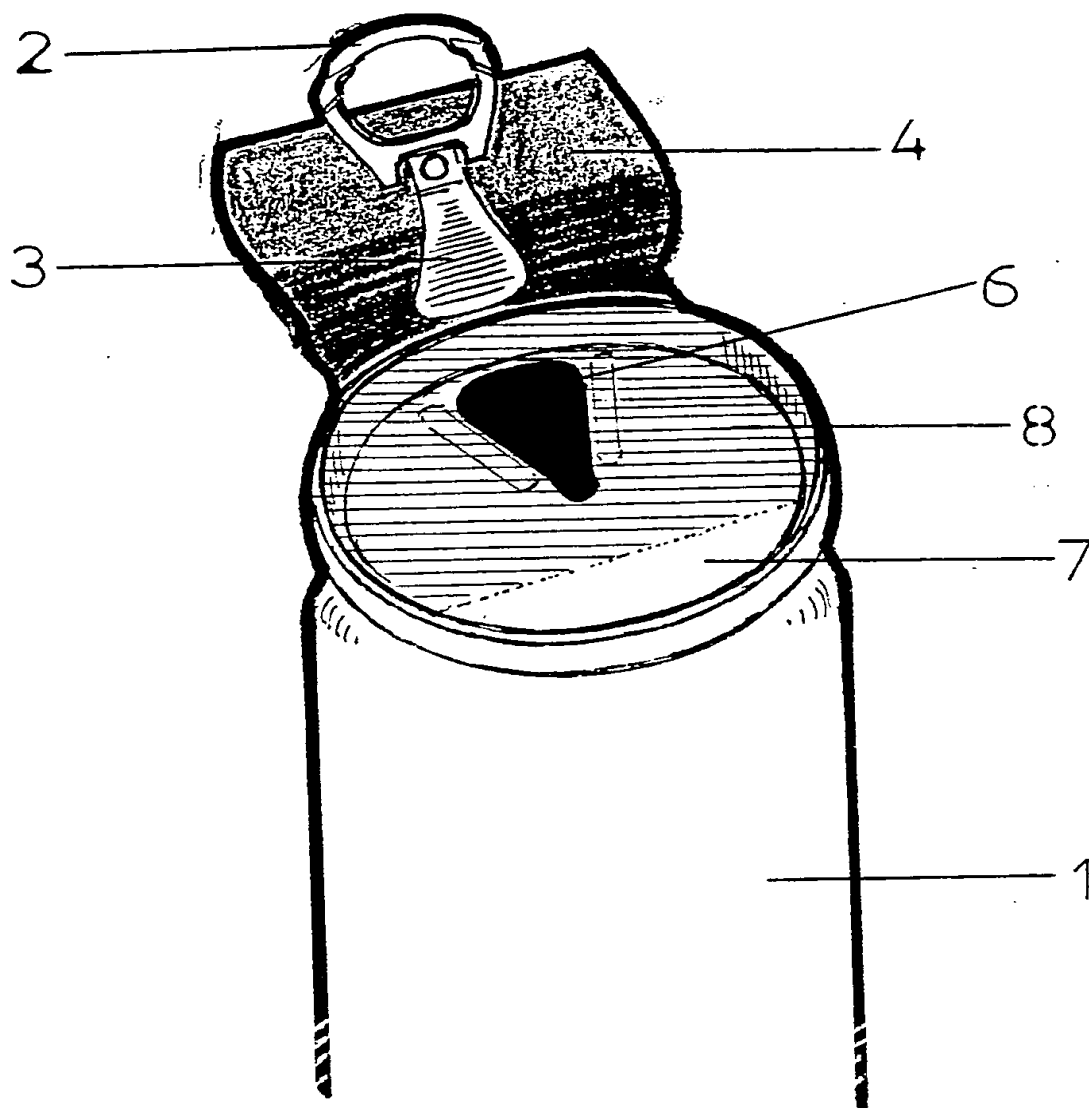
FIGURE 3





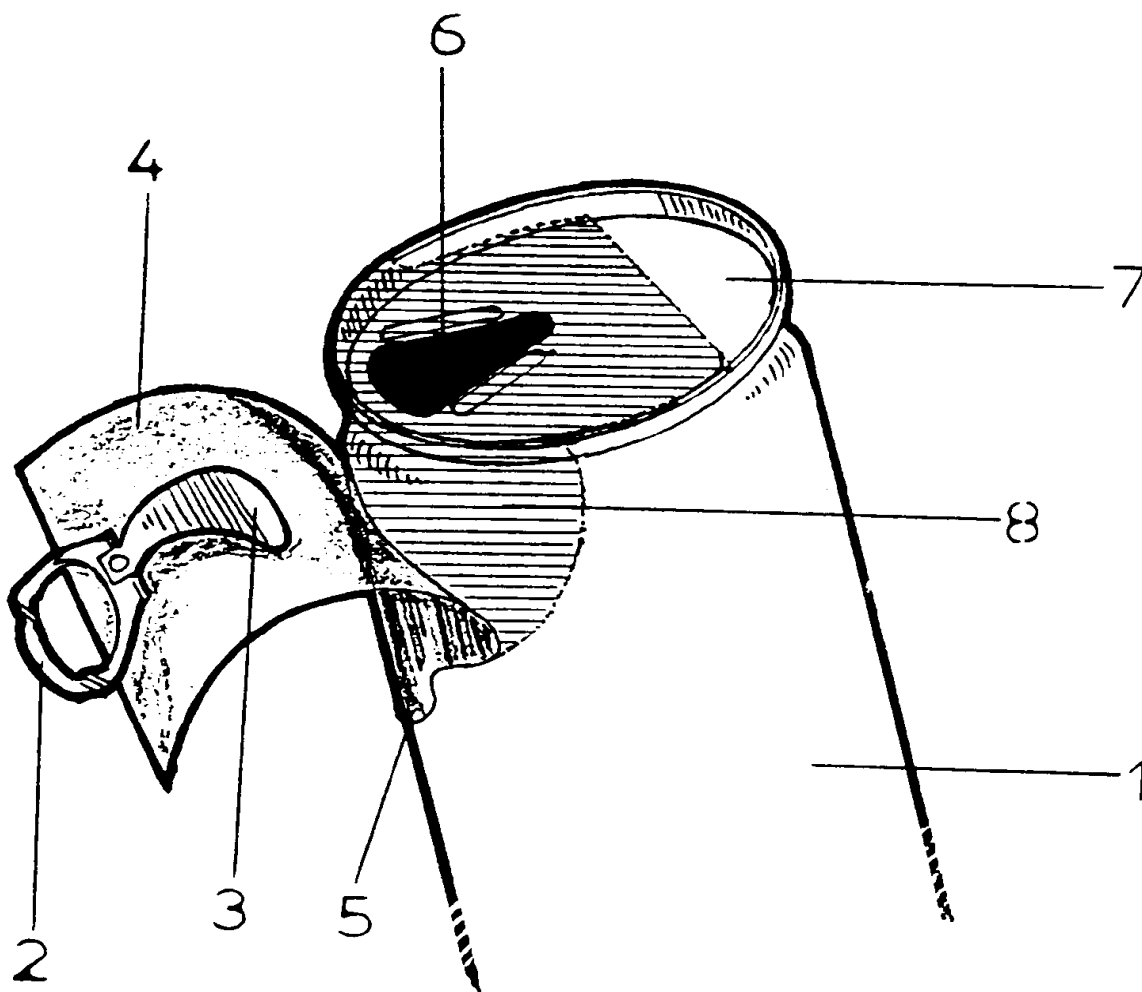
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FIGURE 4



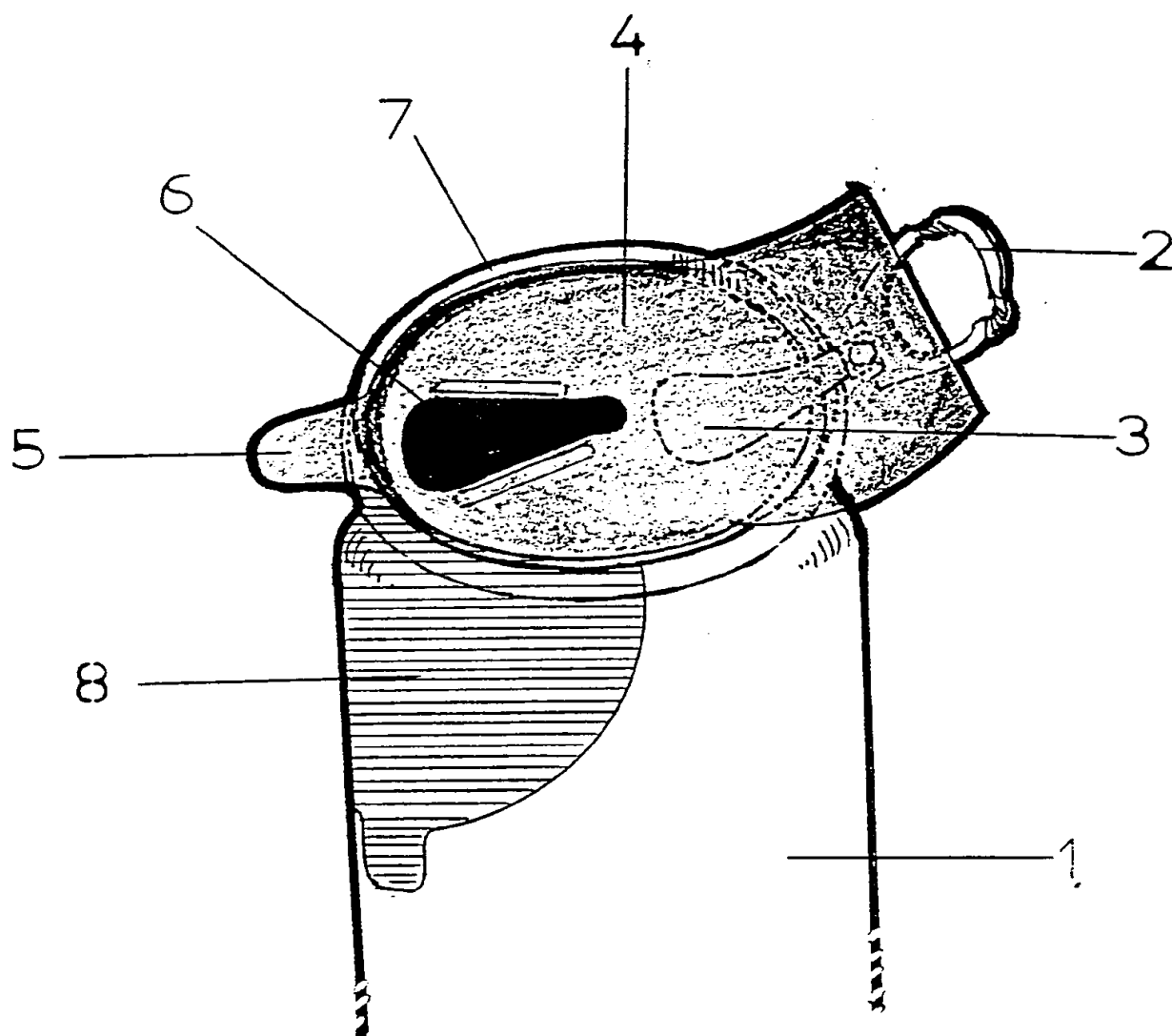
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FIGURE 5



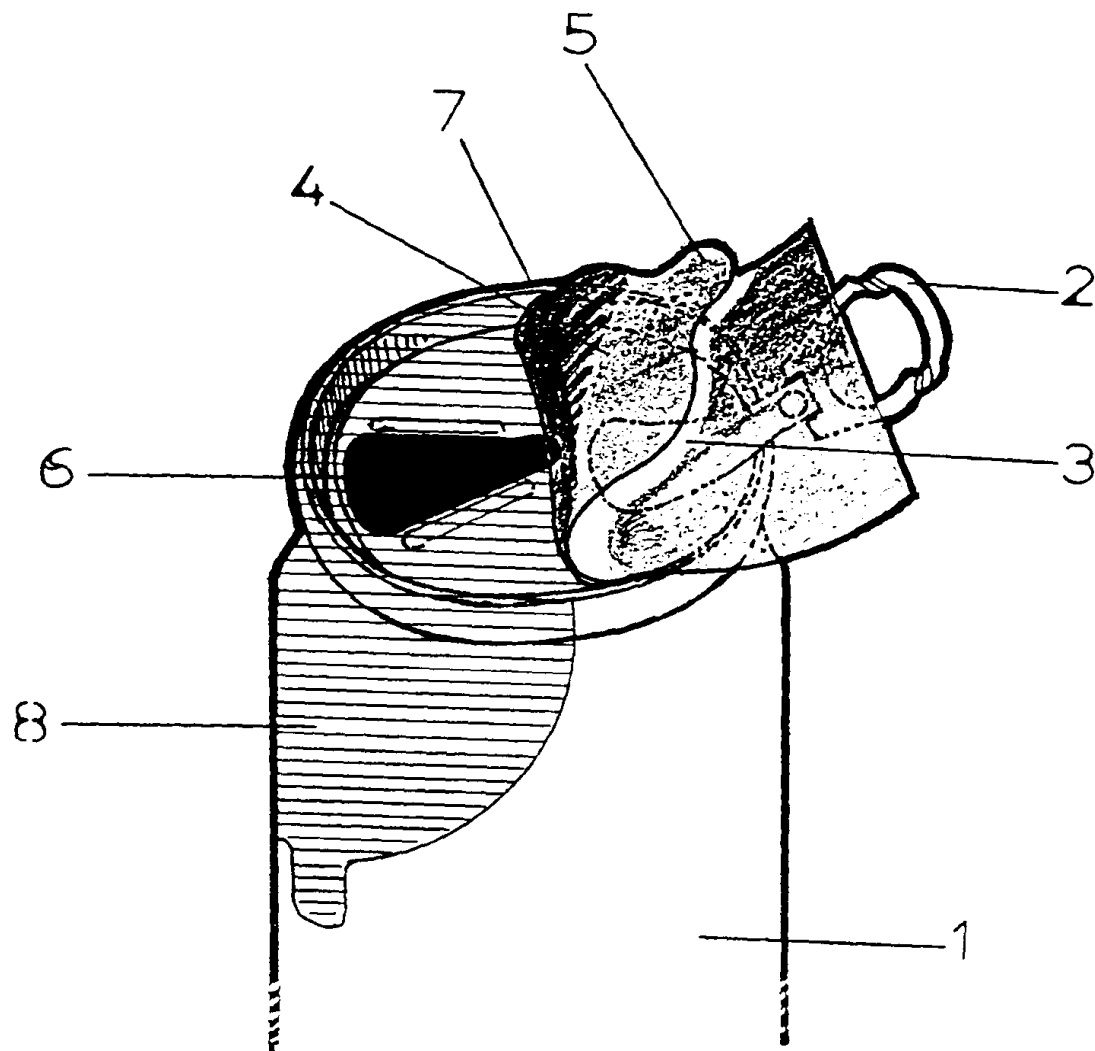
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FIGURE 6



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FIGURE 7



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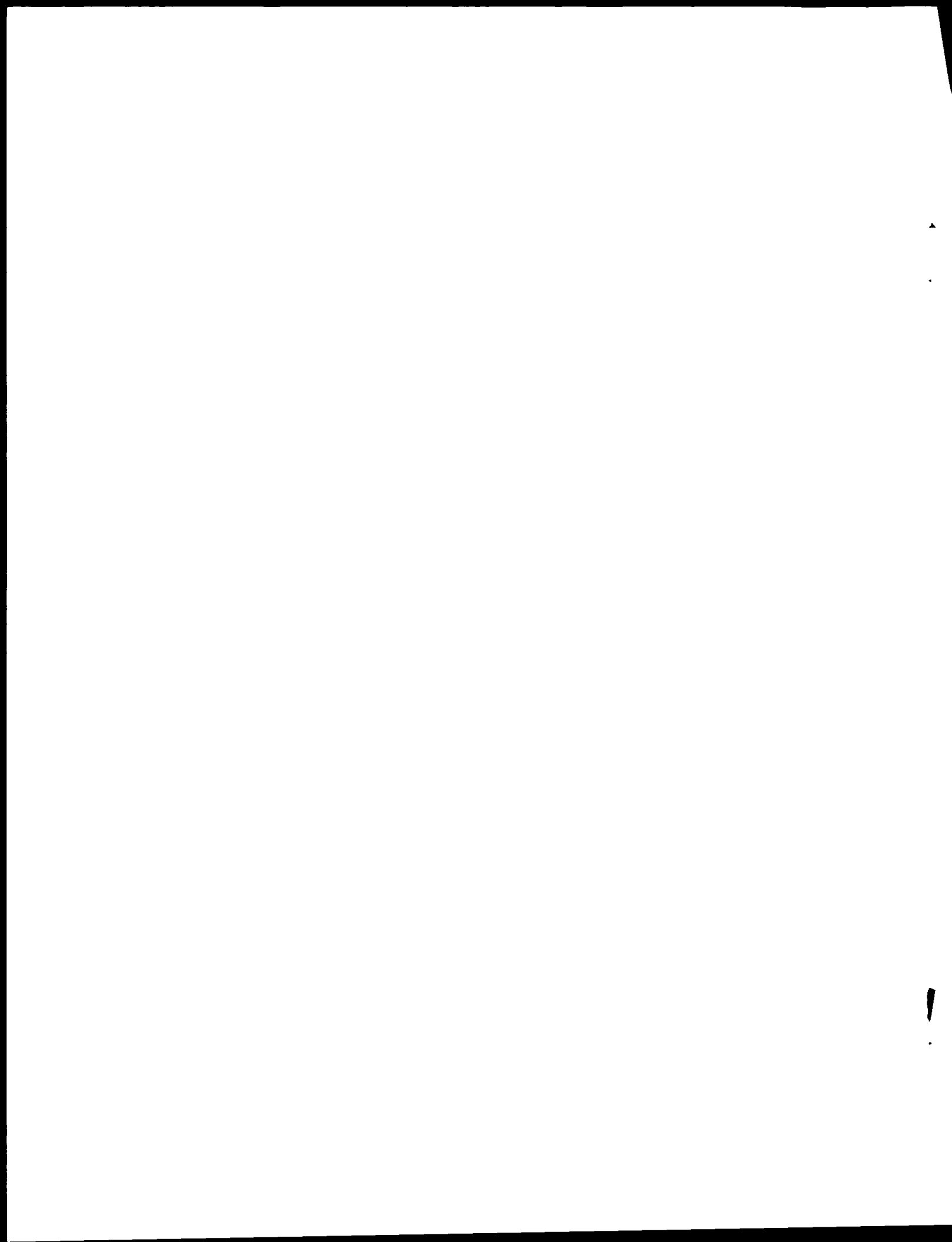
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(54) Title: HUMAN SYNTHETASES

(57) Abstract: The invention provides human synthetases (SYNT) and polynucleotides which identify and encode SYNT. The invention also provides expression vectors, host cells, antibodies, agonists, and antagonists. The invention also provides methods for diagnosing, treating, or preventing disorders associated with expression of SYNT.

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PCT/US 00/19980

## A. CLASSIFICATION OF SUBJECT MATTER

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A61K48/00

According to international Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N C07K C12Q A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

STRAND, EP0-Internal, PAJ, WPI Data

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	NAGASE T ET AL: "PREDICTION OF THE CODING SEQUENCES OF UNIDENTIFIED HUMAN GENES. XVITHE COMPLETE SEQUENCES OF 150 NEW CDNA CLONES FROM BRAIN WHICH CODEFOR LARGE PROTEINS IN VITRO" DNA RESEARCH, UNIVERSAL ACADEMY PRESS, JP, vol. 7, 2000, pages 65-73, XP000949814 ISSN: 1340-2838 SEQUENCE KIAA1352	1-17
P,X	DATABASE EBI [Online] SEQUENCE AC D84223, 15 May 2000 (2000-05-15) H. MOTEKI ET AL.: "Cloning and sequence determination of a human cytoplasmic leucyl-tRNA synthetase gene." XP002152778 sequence data	1-17
	-/-	

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

14 November 2000

Date of mailing of the international search report

19. 02. 01

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# INTERNATIONAL SEARCH REPORT

Inter: [REDACTED] Application No  
PCT/US 00/19980

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 98 16648 A (BALDWIN JACK EDWARD ;CLIFTON IAN (GB); ISIS INNOVATION (GB); ROACH) 23 April 1998 (1998-04-23) the whole document ---	
A	WO 97 40174 A (LEUKOSITE INC) 30 October 1997 (1997-10-30) the whole document ---	
A	US 5 759 833 A (SHIBA KIYOTAKA ET AL) 2 June 1998 (1998-06-02) the whole document -----	



## HUMAN SYNTHETASES

5

### TECHNICAL FIELD

This invention relates to nucleic acid and amino acid sequences of synthetases and to the use of these sequences in the diagnosis, treatment, and prevention of immune, neuronal, and reproductive disorders, and cell proliferative disorders including cancer.

10

### BACKGROUND OF THE INVENTION

A large number of cellular biosynthetic intermediary metabolism processes involve intermolecular transfer of carbon atom-containing substrates (carbon substrates). Examples of such reactions include the tricarboxylic acid cycle, synthesis of fatty acids and long-chain phospholipids, synthesis of alcohols and aldehydes, synthesis of intermediary metabolites, and reactions involved in the amino acid degradation pathways. Many of these reactions are catalyzed by synthetases (also called ligases), which catalyze the formation of a bond between two substrate molecules. Some of these reactions require input of energy, usually in the form of conversion of ATP to either ADP or AMP and pyrophosphate. Synthetases are named for the products of the reaction they catalyze and are involved in such processes as metabolism and the synthesis of macromolecules.

#### 20 Ligases forming carbon-oxygen bonds

Proteins make up more than half of the total dry mass of a cell. The synthesis of proteins is central to cell maintenance, growth, and development. Synthesis occurs on ribosomes and depends on the cooperative interaction of several classes of RNA molecules. The process begins with transcription of the genetic code contained within the DNA to form messenger RNA (mRNA). The mRNA moves in steps through a ribosome and the nucleotide sequence of the mRNA is translated into a corresponding sequence of amino acids to construct a distinct protein chain.

The aminoacyl-transfer RNA (tRNA) synthetases are important RNA-associated enzymes with roles in translation. Protein biosynthesis depends on each amino acid forming a linkage with the appropriate tRNA. The aminoacyl-tRNA synthetases are responsible for the activation and correct attachment of an amino acid with its cognate tRNA. The 20 aminoacyl-tRNA synthetase enzymes can be divided into two structural classes, and each class is characterized by a distinctive topology of the catalytic domain. Class I enzymes contain a catalytic domain based on the nucleotide-binding Rossman 'fold'. Class II enzymes contain a central catalytic domain, which consists of a seven-stranded antiparallel  $\beta$ -sheet motif, as well as N- and C-terminal regulatory domains. Class II enzymes are separated into two groups based on the heterodimeric or homodimeric structure of the enzyme; the latter group is further subdivided by the structure of the N- and C-terminal regulatory

domains (Hartlein, M. and Cusack, S. (1995) *J. Mol. Evol.* 40:519-530). Autoantibodies against aminoacyl-tRNAs are generated by patients with dermatomyositis and polymyositis, and correlate strongly with complicating interstitial lung disease (ILD). These antibodies appear to be generated in response to viral infection, and coxsackie virus has been used to induce experimental viral myositis in animals (Friedman, A.W. et al. (1996) *Semin. Arthritis Rheum.* 26:459-467). A synthetase homolog has been shown to be expressed in chronic myeloid leukemia (CML). A phenylalanine-tRNA synthetase homolog has been found to be tumor-selective and expressed in a cell cycle stage- and differentiation-dependent fashion in an acute-phase human CML cell line (Sen, S. et al. (1997) *Proc. Natl. Acad. Sci. USA* 94:6164-6169).

10 Ligases forming carbon-sulfur bonds (Acid-thiol ligases)

In many cases, a carbon substrate is derived from a small molecule containing at least two carbon atoms. The carbon substrate is often covalently bound to a larger molecule which acts as a carbon substrate carrier molecule within the cell. In the biosynthetic mechanisms described above, the carrier molecule is coenzyme A. Coenzyme A (CoA) is structurally related to derivatives of the nucleotide ADP and consists of 4'-phosphopantetheine linked via a phosphodiester bond to the alpha phosphate group of adenosine 3',5'-bisphosphate. The terminal thiol group of 4'-phosphopantetheine acts as the site for carbon substrate bond formation. The predominant carbon substrates which utilize CoA as a carrier molecule during biosynthesis and intermediary metabolism in the cell are acetyl, succinyl, and propionyl moieties, collectively referred to as acyl groups. Other carbon substrates include enoyl lipid, which acts as a fatty acid oxidation intermediate, and carnitine, which acts as an acetyl-CoA flux regulator/ mitochondrial acyl group transfer protein. Acyl-CoA and acetyl-CoA are synthesized in the cell by acyl-CoA synthetase and acetyl-CoA synthetase, respectively.

Activation of fatty acids is mediated by at least three forms of acyl-CoA synthetase activity: i) acetyl-CoA synthetase, which activates acetate and several other low molecular weight carboxylic acids and is found in muscle mitochondria and the cytosol of other tissues; ii) medium-chain acyl-CoA synthetase, which activates fatty acids containing between four and eleven carbon atoms (predominantly from dietary sources), and is present only in liver mitochondria; and iii) acyl CoA synthetase, which is specific for long chain fatty acids with between six and twenty carbon atoms, and is found in microsomes and the mitochondria. Proteins associated with acyl-CoA synthetase activity have been identified from many sources including bacteria, yeast, plants, mouse, and man. The activity of acyl-CoA synthetase may be modulated by phosphorylation of the enzyme by cAMP-dependent protein kinase. The COL4A5 (collagen, type IV, alpha-5) chromosomal region found deleted in 2 patients with Alport syndrome, elliptocytosis, and mental retardation (Piccini, M. et al. (1998) *Genomics* 47: 350-358) is contiguous with the region containing long-chain acyl-CoA synthetase 4 (FACL4). Therefore, it has been suggested (Piccini supra) that the absence of FACL4 may be involved in the development of mental retardation and other phenotypes associated with

Alport syndrome in these patients.

Ligases forming carbon-nitrogen bonds

A key representative of the amide synthases is the enzyme glutamine synthetase (glutamate-ammonia ligase) that catalyzes the amination of glutamic acid to glutamine by ammonia using the energy of ATP hydrolysis. Glutamine is the primary source for the amino group in various amide transfer reactions involved in de novo pyrimidine nucleotide synthesis and in purine and pyrimidine ribonucleotide interconversions, as well as the conversion of aspartate to asparagine. Overexpression of glutamine synthetase has been observed in primary liver cancer (Christa, L. et al. (1994) Gastroent. 106:1312-1320).

Cyclo-ligases and other carbon-nitrogen ligases comprise various enzymes and enzyme complexes that participate in the de novo pathways to purine and pyrimidine biosynthesis. Because these pathways are critical to the synthesis of nucleotides for replication of both RNA and DNA, many of these enzymes have been the targets of clinical agents for the treatment of cell proliferative disorders such as cancer and infectious diseases.

Purine biosynthesis occurs de novo from the amino acids glycine and glutamine, and other small molecules. Three of the key reactions in this process are catalyzed by a trifunctional enzyme composed of glycinamide-ribonucleotide synthetase (GARS), aminoimidazole ribonucleotide synthetase (AIRS), and glycinamide ribonucleotide transformylase (GART). Together these three enzymes combine ribosylamine phosphate with glycine to yield phosphoribosyl aminoimidazole, a precursor to both adenylate and guanylate nucleotides. This trifunctional protein has been implicated in the pathology of Downs syndrome (Aimi, J. et al. (1990) Nucleic Acid Res. 18:6665-6672). Adenylosuccinate synthetase catalyzes a later step in purine biosynthesis that converts inosinic acid to adenylosuccinate, a key step on the path to ATP synthesis. This enzyme is also similar to another carbon-nitrogen ligase, argininosuccinate synthetase, that catalyzes a similar reaction in the urea cycle (Powell, S.M. et al. (1992) FEBS Lett. 303:4-10).

Like the de novo biosynthesis of purines, de novo synthesis of the pyrimidine nucleotides uridylate and cytidylate also arises from a common precursor, in this instance the nucleotide orotidylate derived from orotate and phosphoribosyl pyrophosphate (PPRP). Again a trifunctional enzyme comprising three carbon-nitrogen ligases plays a key role in the process. In this case the enzymes aspartate transcarbamylase (ATCase), carbamyl phosphate synthetase II, and dihydroorotase (DHOase) are encoded by a single gene called CAD. Together these three enzymes combine the initial reactants in pyrimidine biosynthesis, glutamine, CO<sub>2</sub> and ATP to form dihydroorotate, the precursor to orotate and orotidylate (Iwahana, H. et al. (1996) Biochem. Biophys. Res. Commun. 219:249-255). Further steps then lead to the synthesis of uridine nucleotides from orotidylate.

Cytidine nucleotides are derived from uridine-5'-triphosphate (UTP) by the amidation of UTP using glutamine as the amino donor and the enzyme CTP synthetase. Regulatory mutations in the human

CTP synthetase are believed to confer multi-drug resistance to agents widely used in cancer therapy (Yamauchi, M. et al. (1990) EMBO J. 9:2095-2099).

#### Ligases forming carbon-carbon bonds

Ligases in this group are represented by the carboxylases acetyl-CoA carboxylase and pyruvate carboxylase. Acetyl-CoA carboxylase is a complex which includes a biotin carboxyl carrier protein, biotin carboxylase, and a carboxyl transferase made up of two alpha and two beta subunits. This complex catalyzes the carboxylation of Acetyl-CoA from CO<sub>2</sub> and H<sub>2</sub>O using the energy of ATP hydrolysis (PRINTS document PR01069). Acetyl-CoA carboxylase is the rate-limiting step in the biogenesis of long-chain fatty acids. Two isoforms of Acetyl-CoA carboxylase, types I and types II, are expressed in humans in a tissue-specific manner (Ha, J. et al. (1994) Eur. J. Biochem. 219:297-306). Pyruvate carboxylase is a nuclear-encoded mitochondrial enzyme that catalyzes the conversion of pyruvate to oxaloacetate, a key intermediate in the citric acid cycle.

#### Ligases forming phosphoric ester bonds

Ligases in this group are represented by the DNA ligases involved in both DNA replication and repair. DNA ligases seal phosphodiester bonds between two adjacent nucleotides in a DNA chain using the energy from ATP hydrolysis to first activate the free 5'-phosphate of one nucleotide and then react it with the 3'-OH group of the adjacent nucleotide. This resealing reaction is used in both DNA replication to join small DNA fragments called "Okazaki" fragments that are transiently formed in the process of replicating new DNA, and in DNA repair. DNA repair is the process by which accidental base changes, such as those produced by oxidative damage, hydrolytic attack, or uncontrolled methylation of DNA, are corrected before replication or transcription of the DNA can occur. Bloom's syndrome is an inherited human disease in which individuals are partially deficient in DNA ligation and consequently have an increased incidence of cancer (Alberts, B. et al. (1994) The Molecular Biology of the Cell, Garland Publishing Inc., New York, NY, p. 247).

The discovery of new synthetases and the polynucleotides encoding them satisfies a need in the art by providing new compositions which are useful in the diagnosis, prevention, and treatment of immune, neuronal, and reproductive disorders, and cell proliferative disorders including cancer.

### **SUMMARY OF THE INVENTION**

The invention features purified polypeptides, human synthetases, referred to collectively as "SYNT" and individually as "SYNT-1," "SYNT-2," "SYNT-3," "SYNT-4," "SYNT-5," "SYNT-6," "SYNT-7," "SYNT-8," "SYNT-9," "SYNT-10," "SYNT-11," "SYNT-12," "SYNT-13," "SYNT-14," and "SYNT-15." In one aspect, the invention provides an isolated polypeptide comprising an amino acid sequence selected from the group consisting of a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-15, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID



NO:1-15, c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-15, and d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-15. In one alternative, the invention provides an isolated polypeptide comprising the amino acid sequence of SEQ ID NO:1-15.

5       The invention further provides an isolated polynucleotide encoding a polypeptide comprising an amino acid sequence selected from the group consisting of a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-15, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-15, c) a biologically active fragment of an amino acid sequence selected from the group  
10       consisting of SEQ ID NO:1-15, and d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-15. In one alternative, the polynucleotide encodes a polypeptide selected from the group consisting of SEQ ID NO:1-15. In another alternative, the polynucleotide is selected from the group consisting of SEQ ID NO:16-30.

      Additionally, the invention provides a recombinant polynucleotide comprising a promoter  
15       sequence operably linked to a polynucleotide encoding a polypeptide comprising an amino acid sequence selected from the group consisting of a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-15, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-15, c) a biologically active fragment of an amino acid sequence selected from the group consisting of  
20       SEQ ID NO:1-15, and d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-15. In one alternative, the invention provides a cell transformed with the recombinant polynucleotide. In another alternative, the invention provides a transgenic organism comprising the recombinant polynucleotide.

      The invention also provides a method for producing a polypeptide comprising an amino acid  
25       sequence selected from the group consisting of a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-15, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-15, c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-15, and d) an immunogenic fragment of an amino acid sequence selected from the  
30       group consisting of SEQ ID NO:1-15. The method comprises a) culturing a cell under conditions suitable for expression of the polypeptide, wherein said cell is transformed with a recombinant polynucleotide comprising a promoter sequence operably linked to a polynucleotide encoding the polypeptide, and b) recovering the polypeptide so expressed.

      Additionally, the invention provides an isolated antibody which specifically binds to a  
35       polypeptide comprising an amino acid sequence selected from the group consisting of a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-15, b) a naturally occurring amino

acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-15, c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-15, and d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-15.

5       The invention further provides an isolated polynucleotide comprising a polynucleotide sequence selected from the group consisting of a) a polynucleotide sequence selected from the group consisting of SEQ ID NO:16-30, b) a naturally occurring polynucleotide sequence having at least 90% sequence identity to a polynucleotide sequence selected from the group consisting of SEQ ID NO:16-30, c) a polynucleotide sequence complementary to a), d) a polynucleotide sequence  
10       complementary to b), and e) an RNA equivalent of a)-d). In one alternative, the polynucleotide comprises at least 60 contiguous nucleotides.

          Additionally, the invention provides a method for detecting a target polynucleotide in a sample, said target polynucleotide having a sequence of a polynucleotide comprising a polynucleotide sequence selected from the group consisting of a) a polynucleotide sequence selected from the group  
15       consisting of SEQ ID NO:16-30, b) a naturally occurring polynucleotide sequence having at least 90% sequence identity to a polynucleotide sequence selected from the group consisting of SEQ ID NO:16-30, c) a polynucleotide sequence complementary to a), d) a polynucleotide sequence complementary to b), and e) an RNA equivalent of a)-d). The method comprises a) hybridizing the sample with a probe comprising at least 20 contiguous nucleotides comprising a sequence  
20       complementary to said target polynucleotide in the sample, and which probe specifically hybridizes to said target polynucleotide, under conditions whereby a hybridization complex is formed between said probe and said target polynucleotide or fragments thereof, and b) detecting the presence or absence of said hybridization complex, and optionally, if present, the amount thereof. In one alternative, the probe comprises at least 60 contiguous nucleotides.

25       The invention further provides a method for detecting a target polynucleotide in a sample, said target polynucleotide having a sequence of a polynucleotide comprising a polynucleotide sequence selected from the group consisting of a) a polynucleotide sequence selected from the group consisting of SEQ ID NO:16-30, b) a naturally occurring polynucleotide sequence having at least 90% sequence identity to a polynucleotide sequence selected from the group consisting of SEQ ID  
30       NO:16-30, c) a polynucleotide sequence complementary to a), d) a polynucleotide sequence complementary to b), and e) an RNA equivalent of a)-d). The method comprises a) amplifying said target polynucleotide or fragment thereof using polymerase chain reaction amplification, and b) detecting the presence or absence of said amplified target polynucleotide or fragment thereof, and, optionally, if present, the amount thereof.

35       The invention further provides a pharmaceutical composition comprising an effective amount of a polypeptide comprising an amino acid sequence selected from the group consisting of a) an

amino acid sequence selected from the group consisting of SEQ ID NO:1-15, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-15, c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-15, and d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-15, and a pharmaceutically acceptable excipient. In one embodiment, the pharmaceutical composition comprises an amino acid sequence selected from the group consisting of SEQ ID NO:1-15. The invention additionally provides a method of treating a disease or condition associated with decreased expression of functional SYNT, comprising administering to a patient in need of such treatment the pharmaceutical composition.

The invention also provides a method for screening a compound for effectiveness as an agonist of a polypeptide comprising an amino acid sequence selected from the group consisting of a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-15, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-15, c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-15, and d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-15. The method comprises a) exposing a sample comprising the polypeptide to a compound, and b) detecting agonist activity in the sample. In one alternative, the invention provides a pharmaceutical composition comprising an agonist compound identified by the method and a pharmaceutically acceptable excipient. In another alternative, the invention provides a method of treating a disease or condition associated with decreased expression of functional SYNT, comprising administering to a patient in need of such treatment the pharmaceutical composition.

Additionally, the invention provides a method for screening a compound for effectiveness as an antagonist of a polypeptide comprising an amino acid sequence selected from the group consisting of a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-15, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-15, c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-15, and d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-15. The method comprises a) exposing a sample comprising the polypeptide to a compound, and b) detecting antagonist activity in the sample. In one alternative, the invention provides a pharmaceutical composition comprising an antagonist compound identified by the method and a pharmaceutically acceptable excipient. In another alternative, the invention provides a method of treating a disease or condition associated with overexpression of functional SYNT, comprising administering to a patient in need of such treatment the pharmaceutical composition.

The invention further provides a method of screening for a compound that specifically binds to a polypeptide comprising an amino acid sequence selected from the group consisting of a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-15, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-15, c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-15, and d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-15. The method comprises a) combining the polypeptide with at least one test compound under suitable conditions, and b) detecting binding of the polypeptide to the test compound, thereby identifying a compound that specifically binds to the polypeptide.

The invention further provides a method of screening for a compound that modulates the activity of a polypeptide comprising an amino acid sequence selected from the group consisting of a) an amino acid sequence selected from the group consisting of SEQ ID NO:1-15, b) a naturally occurring amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1-15, c) a biologically active fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-15, and d) an immunogenic fragment of an amino acid sequence selected from the group consisting of SEQ ID NO:1-15. The method comprises a) combining the polypeptide with at least one test compound under conditions permissive for the activity of the polypeptide, b) assessing the activity of the polypeptide in the presence of the test compound, and c) comparing the activity of the polypeptide in the presence of the test compound with the activity of the polypeptide in the absence of the test compound, wherein a change in the activity of the polypeptide in the presence of the test compound is indicative of a compound that modulates the activity of the polypeptide.

The invention further provides a method for screening a compound for effectiveness in altering expression of a target polynucleotide, wherein said target polynucleotide comprises a sequence selected from the group consisting of SEQ ID NO:16-30, the method comprising a) exposing a sample comprising the target polynucleotide to a compound, and b) detecting altered expression of the target polynucleotide.

The invention further provides a method for assessing toxicity of a test compound, said method comprising a) treating a biological sample containing nucleic acids with the test compound; b) hybridizing the nucleic acids of the treated biological sample with a probe comprising at least 20 contiguous nucleotides of a polynucleotide comprising a polynucleotide sequence selected from the group consisting of i) a polynucleotide sequence selected from the group consisting of SEQ ID NO:16-30, ii) a naturally occurring polynucleotide sequence having at least 90% sequence identity to a polynucleotide sequence selected from the group consisting of SEQ ID NO:16-30, iii) a polynucleotide sequence complementary to i), iv) a polynucleotide sequence complementary to ii),

and v) an RNA equivalent of i)-iv). Hybridization occurs under conditions whereby a specific hybridization complex is formed between said probe and a target polynucleotide in the biological sample, said target polynucleotide comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO:16-30, ii) a naturally occurring polynucleotide sequence having at least  
5 90% sequence identity to a polynucleotide sequence selected from the group consisting of SEQ ID NO:16-30, iii) a polynucleotide sequence complementary to i), iv) a polynucleotide sequence complementary to ii), and v) an RNA equivalent of i)-iv). Alternatively, the target polynucleotide comprises a fragment of the above polynucleotide sequence; c) quantifying the amount of hybridization complex; and d) comparing the amount of hybridization complex in the treated  
10 biological sample with the amount of hybridization complex in an untreated biological sample, wherein a difference in the amount of hybridization complex in the treated biological sample is indicative of toxicity of the test compound.

### BRIEF DESCRIPTION OF THE TABLES

15 Table 1 shows polypeptide and nucleotide sequence identification numbers (SEQ ID NOs), clone identification numbers (clone IDs), cDNA libraries, and cDNA fragments used to assemble full-length sequences encoding SYNT.

Table 2 shows features of each polypeptide sequence, including potential motifs, homologous sequences, and methods, algorithms, and searchable databases used for analysis of SYNT.

20 Table 3 shows selected fragments of each nucleic acid sequence; the tissue-specific expression patterns of each nucleic acid sequence as determined by northern analysis; diseases, disorders, or conditions associated with these tissues; and the vector into which each cDNA was cloned.

Table 4 describes the tissues used to construct the cDNA libraries from which cDNA clones  
25 encoding SYNT were isolated.

Table 5 shows the tools, programs, and algorithms used to analyze the polynucleotides and polypeptides of the invention, along with applicable descriptions, references, and threshold parameters.

### DESCRIPTION OF THE INVENTION

30 Before the present proteins, nucleotide sequences, and methods are described, it is understood that this invention is not limited to the particular machines, materials and methods described, as these may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which  
35 will be limited only by the appended claims.

It must be noted that as used herein and in the appended claims, the singular forms "a," "an,"

and "the" include plural reference unless the context clearly dictates otherwise. Thus, for example, a reference to "a host cell" includes a plurality of such host cells, and a reference to "an antibody" is a reference to one or more antibodies and equivalents thereof known to those skilled in the art, and so forth.

5 Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any machines, materials, and methods similar or equivalent to those described herein can be used to practice or test the present invention, the preferred machines, materials and methods are now described. All publications mentioned herein are cited for the purpose of describing and disclosing  
10 the cell lines, protocols, reagents and vectors which are reported in the publications and which might be used in connection with the invention. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

### DEFINITIONS

"SYNT" refers to the amino acid sequences of substantially purified SYNT obtained from  
15 any species, particularly a mammalian species, including bovine, ovine, porcine, murine, equine, and human, and from any source, whether natural, synthetic, semi-synthetic, or recombinant.

The term "agonist" refers to a molecule which intensifies or mimics the biological activity of SYNT. Agonists may include proteins, nucleic acids, carbohydrates, small molecules, or any other compound or composition which modulates the activity of SYNT either by directly interacting with  
20 SYNT or by acting on components of the biological pathway in which SYNT participates.

An "allelic variant" is an alternative form of the gene encoding SYNT. Allelic variants may result from at least one mutation in the nucleic acid sequence and may result in altered mRNAs or in polypeptides whose structure or function may or may not be altered. A gene may have none, one, or many allelic variants of its naturally occurring form. Common mutational changes which give rise to  
25 allelic variants are generally ascribed to natural deletions, additions, or substitutions of nucleotides. Each of these types of changes may occur alone, or in combination with the others, one or more times in a given sequence.

"Altered" nucleic acid sequences encoding SYNT include those sequences with deletions, insertions, or substitutions of different nucleotides, resulting in a polypeptide the same as SYNT or a  
30 polypeptide with at least one functional characteristic of SYNT. Included within this definition are polymorphisms which may or may not be readily detectable using a particular oligonucleotide probe of the polynucleotide encoding SYNT, and improper or unexpected hybridization to allelic variants, with a locus other than the normal chromosomal locus for the polynucleotide sequence encoding SYNT. The encoded protein may also be "altered," and may contain deletions, insertions, or  
35 substitutions of amino acid residues which produce a silent change and result in a functionally equivalent SYNT. Deliberate amino acid substitutions may be made on the basis of similarity in

polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues, as long as the biological or immunological activity of SYNT is retained. For example, negatively charged amino acids may include aspartic acid and glutamic acid, and positively charged amino acids may include lysine and arginine. Amino acids with uncharged polar side chains having similar hydrophilicity values may include: asparagine and glutamine; and serine and threonine. Amino acids with uncharged side chains having similar hydrophilicity values may include: leucine, isoleucine, and valine; glycine and alanine; and phenylalanine and tyrosine.

The terms "amino acid" and "amino acid sequence" refer to an oligopeptide, peptide, polypeptide, or protein sequence, or a fragment of any of these, and to naturally occurring or synthetic molecules. Where "amino acid sequence" is recited to refer to a sequence of a naturally occurring protein molecule, "amino acid sequence" and like terms are not meant to limit the amino acid sequence to the complete native amino acid sequence associated with the recited protein molecule.

"Amplification" relates to the production of additional copies of a nucleic acid sequence. Amplification is generally carried out using polymerase chain reaction (PCR) technologies well known in the art.

The term "antagonist" refers to a molecule which inhibits or attenuates the biological activity of SYNT. Antagonists may include proteins such as antibodies, nucleic acids, carbohydrates, small molecules, or any other compound or composition which modulates the activity of SYNT either by directly interacting with SYNT or by acting on components of the biological pathway in which SYNT participates.

The term "antibody" refers to intact immunoglobulin molecules as well as to fragments thereof, such as Fab, F(ab')<sub>2</sub>, and Fv fragments, which are capable of binding an epitopic determinant. Antibodies that bind SYNT polypeptides can be prepared using intact polypeptides or using fragments containing small peptides of interest as the immunizing antigen. The polypeptide or oligopeptide used to immunize an animal (e.g., a mouse, a rat, or a rabbit) can be derived from the translation of RNA, or synthesized chemically, and can be conjugated to a carrier protein if desired. Commonly used carriers that are chemically coupled to peptides include bovine serum albumin, thyroglobulin, and keyhole limpet hemocyanin (KLH). The coupled peptide is then used to immunize the animal.

The term "antigenic determinant" refers to that region of a molecule (i.e., an epitope) that makes contact with a particular antibody. When a protein or a fragment of a protein is used to immunize a host animal, numerous regions of the protein may induce the production of antibodies which bind specifically to antigenic determinants (particular regions or three-dimensional structures on the protein). An antigenic determinant may compete with the intact antigen (i.e., the immunogen used to elicit the immune response) for binding to an antibody.

The term "antisense" refers to any composition capable of base-pairing with the "sense"

(coding) strand of a specific nucleic acid sequence. Antisense compositions may include DNA; RNA; peptide nucleic acid (PNA); oligonucleotides having modified backbone linkages such as phosphorothioates, methylphosphonates, or benzylphosphonates; oligonucleotides having modified sugar groups such as 2'-methoxyethyl sugars or 2'-methoxyethoxy sugars; or oligonucleotides having modified bases such as 5-methyl cytosine, 2'-deoxyuracil, or 7-deaza-2'-deoxyguanosine. Antisense molecules may be produced by any method including chemical synthesis or transcription. Once introduced into a cell, the complementary antisense molecule base-pairs with a naturally occurring nucleic acid sequence produced by the cell to form duplexes which block either transcription or translation. The designation "negative" or "minus" can refer to the antisense strand, and the designation "positive" or "plus" can refer to the sense strand of a reference DNA molecule.

The term "biologically active" refers to a protein having structural, regulatory, or biochemical functions of a naturally occurring molecule. Likewise, "immunologically active" or "immunogenic" refers to the capability of the natural, recombinant, or synthetic SYNT, or of any oligopeptide thereof, to induce a specific immune response in appropriate animals or cells and to bind with specific antibodies.

"Complementary" describes the relationship between two single-stranded nucleic acid sequences that anneal by base-pairing. For example, 5'-AGT-3' pairs with its complement, 3'-TCA-5'.

A "composition comprising a given polynucleotide sequence" and a "composition comprising a given amino acid sequence" refer broadly to any composition containing the given polynucleotide or amino acid sequence. The composition may comprise a dry formulation or an aqueous solution. Compositions comprising polynucleotide sequences encoding SYNT or fragments of SYNT may be employed as hybridization probes. The probes may be stored in freeze-dried form and may be associated with a stabilizing agent such as a carbohydrate. In hybridizations, the probe may be deployed in an aqueous solution containing salts (e.g., NaCl), detergents (e.g., sodium dodecyl sulfate; SDS), and other components (e.g., Denhardt's solution, dry milk, salmon sperm DNA, etc.).

"Consensus sequence" refers to a nucleic acid sequence which has been subjected to repeated DNA sequence analysis to resolve uncalled bases, extended using the XL-PCR kit (PE Biosystems, Foster City CA) in the 5' and/or the 3' direction, and resequenced, or which has been assembled from one or more overlapping cDNA, EST, or genomic DNA fragments using a computer program for fragment assembly, such as the GELVIEW fragment assembly system (GCG, Madison WI) or Phrap (University of Washington, Seattle WA). Some sequences have been both extended and assembled to produce the consensus sequence.

"Conservative amino acid substitutions" are those substitutions that are predicted to least interfere with the properties of the original protein, i.e., the structure and especially the function of the protein is conserved and not significantly changed by such substitutions. The table below shows



amino acids which may be substituted for an original amino acid in a protein and which are regarded as conservative amino acid substitutions.

	Original Residue	Conservative Substitution
5	Ala	Gly, Ser
	Arg	His, Lys
	Asn	Asp, Gln, His
	Asp	Asn, Glu
	Cys	Ala, Ser
10	Gln	Asn, Glu, His
	Glu	Asp, Gln, His
	Gly	Ala
	His	Asn, Arg, Gln, Glu
	Ile	Leu, Val
15	Leu	Ile, Val
	Lys	Arg, Gln, Glu
	Met	Leu, Ile
	Phe	His, Met, Leu, Trp, Tyr
	Ser	Cys, Thr
20	Thr	Ser, Val
	Trp	Phe, Tyr
	Tyr	His, Phe, Trp
	Val	Ile, Leu, Thr

Conservative amino acid substitutions generally maintain (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a beta sheet or alpha helical conformation, (b) the charge or hydrophobicity of the molecule at the site of the substitution, and/or (c) the bulk of the side chain.

A "deletion" refers to a change in the amino acid or nucleotide sequence that results in the absence of one or more amino acid residues or nucleotides.

The term "derivative" refers to a chemically modified polynucleotide or polypeptide. Chemical modifications of a polynucleotide sequence can include, for example, replacement of hydrogen by an alkyl, acyl, hydroxyl, or amino group. A derivative polynucleotide encodes a polypeptide which retains at least one biological or immunological function of the natural molecule. A derivative polypeptide is one modified by glycosylation, pegylation, or any similar process that retains at least one biological or immunological function of the polypeptide from which it was derived.

A "detectable label" refers to a reporter molecule or enzyme that is capable of generating a measurable signal and is covalently or noncovalently joined to a polynucleotide or polypeptide.

A "fragment" is a unique portion of SYNT or the polynucleotide encoding SYNT which is identical in sequence to but shorter in length than the parent sequence. A fragment may comprise up to the entire length of the defined sequence, minus one nucleotide/amino acid residue. For example, a fragment may comprise from 5 to 1000 contiguous nucleotides or amino acid residues. A fragment used as a probe, primer, antigen, therapeutic molecule, or for other purposes, may be at least 5, 10,

15, 16, 20, 25, 30, 40, 50, 60, 75, 100, 150, 250 or at least 500 contiguous nucleotides or amino acid residues in length. Fragments may be preferentially selected from certain regions of a molecule. For example, a polypeptide fragment may comprise a certain length of contiguous amino acids selected from the first 250 or 500 amino acids (or first 25% or 50% of a polypeptide) as shown in a certain defined sequence. Clearly these lengths are exemplary, and any length that is supported by the specification, including the Sequence Listing, tables, and figures, may be encompassed by the present embodiments.

A fragment of SEQ ID NO:16-30 comprises a region of unique polynucleotide sequence that specifically identifies SEQ ID NO:16-30, for example, as distinct from any other sequence in the genome from which the fragment was obtained. A fragment of SEQ ID NO:16-30 is useful, for example, in hybridization and amplification technologies and in analogous methods that distinguish SEQ ID NO:16-30 from related polynucleotide sequences. The precise length of a fragment of SEQ ID NO:16-30 and the region of SEQ ID NO:16-30 to which the fragment corresponds are routinely determinable by one of ordinary skill in the art based on the intended purpose for the fragment.

A fragment of SEQ ID NO:1-15 is encoded by a fragment of SEQ ID NO:16-30. A fragment of SEQ ID NO:1-15 comprises a region of unique amino acid sequence that specifically identifies SEQ ID NO:1-15. For example, a fragment of SEQ ID NO:1-15 is useful as an immunogenic peptide for the development of antibodies that specifically recognize SEQ ID NO:1-15. The precise length of a fragment of SEQ ID NO:1-15 and the region of SEQ ID NO:1-15 to which the fragment corresponds are routinely determinable by one of ordinary skill in the art based on the intended purpose for the fragment.

A "full-length" polynucleotide sequence is one containing at least a translation initiation codon (e.g., methionine) followed by an open reading frame and a translation termination codon. A "full-length" polynucleotide sequence encodes a "full-length" polypeptide sequence.

"Homology" refers to sequence similarity or, interchangeably, sequence identity, between two or more polynucleotide sequences or two or more polypeptide sequences.

The terms "percent identity" and "% identity," as applied to polynucleotide sequences, refer to the percentage of residue matches between at least two polynucleotide sequences aligned using a standardized algorithm. Such an algorithm may insert, in a standardized and reproducible way, gaps in the sequences being compared in order to optimize alignment between two sequences, and therefore achieve a more meaningful comparison of the two sequences.

Percent identity between polynucleotide sequences may be determined using the default parameters of the CLUSTAL V algorithm as incorporated into the MEGALIGN version 3.12e sequence alignment program. This program is part of the LASERGENE software package, a suite of molecular biological analysis programs (DNASTAR, Madison WI). CLUSTAL V is described in Higgins, D.G. and P.M. Sharp (1989) CABIOS 5:151-153 and in Higgins, D.G. et al. (1992) CABIOS

8:189-191. For pairwise alignments of polynucleotide sequences, the default parameters are set as follows: Ktuple=2, gap penalty=5, window=4, and "diagonals saved"=4. The "weighted" residue weight table is selected as the default. Percent identity is reported by CLUSTAL V as the "percent similarity" between aligned polynucleotide sequences.

5           Alternatively, a suite of commonly used and freely available sequence comparison algorithms is provided by the National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST) (Altschul, S.F. et al. (1990) J. Mol. Biol. 215:403-410), which is available from several sources, including the NCBI, Bethesda, MD, and on the Internet at <http://www.ncbi.nlm.nih.gov/BLAST/>. The BLAST software suite includes various sequence  
10   analysis programs including "blastn," that is used to align a known polynucleotide sequence with other polynucleotide sequences from a variety of databases. Also available is a tool called "BLAST 2 Sequences" that is used for direct pairwise comparison of two nucleotide sequences. "BLAST 2 Sequences" can be accessed and used interactively at <http://www.ncbi.nlm.nih.gov/gorf/bl2.html>. The "BLAST 2 Sequences" tool can be used for both blastn and blastp (discussed below). BLAST  
15   programs are commonly used with gap and other parameters set to default settings. For example, to compare two nucleotide sequences, one may use blastn with the "BLAST 2 Sequences" tool Version 2.0.12 (April-21-2000) set at default parameters. Such default parameters may be, for example:

*Matrix: BLOSUM62*

*Reward for match: 1*

20   *Penalty for mismatch: -2*

*Open Gap: 5 and Extension Gap: 2 penalties*

*Gap x drop-off: 50*

*Expect: 10*

*Word Size: 11*

25   *Filter: on*

Percent identity may be measured over the length of an entire defined sequence, for example, as defined by a particular SEQ ID number, or may be measured over a shorter length, for example, over the length of a fragment taken from a larger, defined sequence, for instance, a fragment of at least 20, at least 30, at least 40, at least 50, at least 70, at least 100, or at least 200 contiguous  
30   nucleotides. Such lengths are exemplary only, and it is understood that any fragment length supported by the sequences shown herein, in the tables, figures, or Sequence Listing, may be used to describe a length over which percentage identity may be measured.

Nucleic acid sequences that do not show a high degree of identity may nevertheless encode similar amino acid sequences due to the degeneracy of the genetic code. It is understood that changes  
35   in a nucleic acid sequence can be made using this degeneracy to produce multiple nucleic acid sequences that all encode substantially the same protein.

The phrases "percent identity" and "% identity," as applied to polypeptide sequences, refer to the percentage of residue matches between at least two polypeptide sequences aligned using a standardized algorithm. Methods of polypeptide sequence alignment are well-known. Some alignment methods take into account conservative amino acid substitutions. Such conservative substitutions, explained in more detail above, generally preserve the charge and hydrophobicity at the site of substitution, thus preserving the structure (and therefore function) of the polypeptide.

Percent identity between polypeptide sequences may be determined using the default parameters of the CLUSTAL V algorithm as incorporated into the MEGALIGN version 3.12e sequence alignment program (described and referenced above). For pairwise alignments of polypeptide sequences using CLUSTAL V, the default parameters are set as follows: Ktuple=1, gap penalty=3, window=5, and "diagonals saved"=5. The PAM250 matrix is selected as the default residue weight table. As with polynucleotide alignments, the percent identity is reported by CLUSTAL V as the "percent similarity" between aligned polypeptide sequence pairs.

Alternatively the NCBI BLAST software suite may be used. For example, for a pairwise comparison of two polypeptide sequences, one may use the "BLAST 2 Sequences" tool Version 2.0.12 (Apr-21-2000) with blastp set at default parameters. Such default parameters may be, for example:

*Matrix: BLOSUM62*

*Open Gap: 11 and Extension Gap: 1 penalties*

*Gap x drop-off: 50*

*Expect: 10*

*Word Size: 3*

*Filter: on*

Percent identity may be measured over the length of an entire defined polypeptide sequence, for example, as defined by a particular SEQ ID number, or may be measured over a shorter length, for example, over the length of a fragment taken from a larger, defined polypeptide sequence, for instance, a fragment of at least 15, at least 20, at least 30, at least 40, at least 50, at least 70 or at least 150 contiguous residues. Such lengths are exemplary only, and it is understood that any fragment length supported by the sequences shown herein, in the tables, figures or Sequence Listing, may be used to describe a length over which percentage identity may be measured.

"Human artificial chromosomes" (HACs) are linear microchromosomes which may contain DNA sequences of about 6 kb to 10 Mb in size, and which contain all of the elements required for chromosome replication, segregation and maintenance.

The term "humanized antibody" refers to an antibody molecule in which the amino acid sequence in the non-antigen binding regions has been altered so that the antibody more closely resembles a human antibody, and still retains its original binding ability.

"Hybridization" refers to the process by which a polynucleotide strand anneals with a complementary strand through base pairing under defined hybridization conditions. Specific hybridization is an indication that two nucleic acid sequences share a high degree of complementarity. Specific hybridization complexes form under permissive annealing conditions and remain hybridized after the "washing" step(s). The washing step(s) is particularly important in determining the stringency of the hybridization process, with more stringent conditions allowing less non-specific binding, i.e., binding between pairs of nucleic acid strands that are not perfectly matched. Permissive conditions for annealing of nucleic acid sequences are routinely determinable by one of ordinary skill in the art and may be consistent among hybridization experiments, whereas wash conditions may be varied among experiments to achieve the desired stringency, and therefore hybridization specificity. Permissive annealing conditions occur, for example, at 68°C in the presence of about 6 x SSC, about 1% (w/v) SDS, and about 100 µg/ml sheared, denatured salmon sperm DNA.

Generally, stringency of hybridization is expressed, in part, with reference to the temperature under which the wash step is carried out. Such wash temperatures are typically selected to be about 5°C to 20°C lower than the thermal melting point ( $T_m$ ) for the specific sequence at a defined ionic strength and pH. The  $T_m$  is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. An equation for calculating  $T_m$  and conditions for nucleic acid hybridization are well known and can be found in Sambrook, J. et al., 1989, Molecular Cloning: A Laboratory Manual, 2<sup>nd</sup> ed., vol. 1-3, Cold Spring Harbor Press, Plainview NY; specifically see volume 2, chapter 9.

High stringency conditions for hybridization between polynucleotides of the present invention include wash conditions of 68°C in the presence of about 0.2 x SSC and about 0.1% SDS, for 1 hour. Alternatively, temperatures of about 65°C, 60°C, 55°C, or 42°C may be used. SSC concentration may be varied from about 0.1 to 2 x SSC, with SDS being present at about 0.1%. Typically, blocking reagents are used to block non-specific hybridization. Such blocking reagents include, for instance, sheared and denatured salmon sperm DNA at about 100-200 µg/ml. Organic solvent, such as formamide at a concentration of about 35-50% v/v, may also be used under particular circumstances, such as for RNA:DNA hybridizations. Useful variations on these wash conditions will be readily apparent to those of ordinary skill in the art. Hybridization, particularly under high stringency conditions, may be suggestive of evolutionary similarity between the nucleotides. Such similarity is strongly indicative of a similar role for the nucleotides and their encoded polypeptides.

The term "hybridization complex" refers to a complex formed between two nucleic acid sequences by virtue of the formation of hydrogen bonds between complementary bases. A hybridization complex may be formed in solution (e.g.,  $C_0t$  or  $R_0t$  analysis) or formed between one nucleic acid sequence present in solution and another nucleic acid sequence immobilized on a solid support (e.g., paper, membranes, filters, chips, pins or glass slides, or any other appropriate substrate

to which cells or their nucleic acids have been fixed).

The words "insertion" and "addition" refer to changes in an amino acid or nucleotide sequence resulting in the addition of one or more amino acid residues or nucleotides, respectively.

5 "Immune response" can refer to conditions associated with inflammation, trauma, immune disorders, or infectious or genetic disease, etc. These conditions can be characterized by expression of various factors, e.g., cytokines, chemokines, and other signaling molecules, which may affect cellular and systemic defense systems.

10 An "immunogenic fragment" is a polypeptide or oligopeptide fragment of SYNT which is capable of eliciting an immune response when introduced into a living organism, for example, a mammal. The term "immunogenic fragment" also includes any polypeptide or oligopeptide fragment of SYNT which is useful in any of the antibody production methods disclosed herein or known in the art.

The term "microarray" refers to an arrangement of a plurality of polynucleotides, polypeptides, or other chemical compounds on a substrate.

15 The terms "element" and "array element" refer to a polynucleotide, polypeptide, or other chemical compound having a unique and defined position on a microarray.

The term "modulate" refers to a change in the activity of SYNT. For example, modulation may cause an increase or a decrease in protein activity, binding characteristics, or any other biological, functional, or immunological properties of SYNT.

20 The phrases "nucleic acid" and "nucleic acid sequence" refer to a nucleotide, oligonucleotide, polynucleotide, or any fragment thereof. These phrases also refer to DNA or RNA of genomic or synthetic origin which may be single-stranded or double-stranded and may represent the sense or the antisense strand, to peptide nucleic acid (PNA), or to any DNA-like or RNA-like material.

25 "Operably linked" refers to the situation in which a first nucleic acid sequence is placed in a functional relationship with a second nucleic acid sequence. For instance, a promoter is operably linked to a coding sequence if the promoter affects the transcription or expression of the coding sequence. Operably linked DNA sequences may be in close proximity or contiguous and, where necessary to join two protein coding regions, in the same reading frame.

30 "Peptide nucleic acid" (PNA) refers to an antisense molecule or anti-gene agent which comprises an oligonucleotide of at least about 5 nucleotides in length linked to a peptide backbone of amino acid residues ending in lysine. The terminal lysine confers solubility to the composition. PNAs preferentially bind complementary single stranded DNA or RNA and stop transcript elongation, and may be pegylated to extend their lifespan in the cell.

35 "Post-translational modification" of an SYNT may involve lipidation, glycosylation, phosphorylation, acetylation, racemization, proteolytic cleavage, and other modifications known in the art. These processes may occur synthetically or biochemically. Biochemical modifications will

vary by cell type depending on the enzymatic milieu of SYNT.

"Probe" refers to nucleic acid sequences encoding SYNT, their complements, or fragments thereof, which are used to detect identical, allelic or related nucleic acid sequences. Probes are isolated oligonucleotides or polynucleotides attached to a detectable label or reporter molecule.

5 Typical labels include radioactive isotopes, ligands, chemiluminescent agents, and enzymes.

"Primers" are short nucleic acids, usually DNA oligonucleotides, which may be annealed to a target polynucleotide by complementary base-pairing. The primer may then be extended along the target DNA strand by a DNA polymerase enzyme. Primer pairs can be used for amplification (and identification) of a nucleic acid sequence, e.g., by the polymerase chain reaction (PCR).

10 Probes and primers as used in the present invention typically comprise at least 15 contiguous nucleotides of a known sequence. In order to enhance specificity, longer probes and primers may also be employed, such as probes and primers that comprise at least 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, or at least 150 consecutive nucleotides of the disclosed nucleic acid sequences. Probes and primers may be considerably longer than these examples, and it is understood that any length supported by the  
15 specification, including the tables, figures, and Sequence Listing, may be used.

Methods for preparing and using probes and primers are described in the references, for example Sambrook, J. et al., 1989, Molecular Cloning: A Laboratory Manual, 2<sup>nd</sup> ed., vol. 1-3, Cold Spring Harbor Press, Plainview NY; Ausubel, F.M. et al., 1987, Current Protocols in Molecular Biology, Greene Publ. Assoc. & Wiley-Intersciences, New York NY; Innis, M. et al., 1990, PCR  
20 Protocols, A Guide to Methods and Applications, Academic Press, San Diego CA. PCR primer pairs can be derived from a known sequence, for example, by using computer programs intended for that purpose such as Primer (Version 0.5, 1991, Whitehead Institute for Biomedical Research, Cambridge MA).

Oligonucleotides for use as primers are selected using software known in the art for such  
25 purpose. For example, OLIGO 4.06 software is useful for the selection of PCR primer pairs of up to 100 nucleotides each, and for the analysis of oligonucleotides and larger polynucleotides of up to 5,000 nucleotides from an input polynucleotide sequence of up to 32 kilobases. Similar primer selection programs have incorporated additional features for expanded capabilities. For example, the PrimOU primer selection program (available to the public from the Genome Center at University of  
30 Texas South West Medical Center, Dallas TX) is capable of choosing specific primers from megabase sequences and is thus useful for designing primers on a genome-wide scope. The Primer3 primer selection program (available to the public from the Whitehead Institute/MIT Center for Genome Research, Cambridge MA) allows the user to input a "mispriming library," in which sequences to avoid as primer binding sites are user-specified. Primer3 is useful, in particular, for the  
35 selection of oligonucleotides for microarrays. (The source code for the latter two primer selection programs may also be obtained from their respective sources and modified to meet the user's specific

needs.) The PrimeGen program (available to the public from the UK Human Genome Mapping Project Resource Centre, Cambridge UK) designs primers based on multiple sequence alignments, thereby allowing selection of primers that hybridize to either the most conserved or least conserved regions of aligned nucleic acid sequences. Hence, this program is useful for identification of both  
5 unique and conserved oligonucleotides and polynucleotide fragments. The oligonucleotides and polynucleotide fragments identified by any of the above selection methods are useful in hybridization technologies, for example, as PCR or sequencing primers, microarray elements, or specific probes to identify fully or partially complementary polynucleotides in a sample of nucleic acids. Methods of oligonucleotide selection are not limited to those described above.

10 A "recombinant nucleic acid" is a sequence that is not naturally occurring or has a sequence that is made by an artificial combination of two or more otherwise separated segments of sequence. This artificial combination is often accomplished by chemical synthesis or, more commonly, by the artificial manipulation of isolated segments of nucleic acids, e.g., by genetic engineering techniques such as those described in Sambrook, supra. The term recombinant includes nucleic acids that have  
15 been altered solely by addition, substitution, or deletion of a portion of the nucleic acid. Frequently, a recombinant nucleic acid may include a nucleic acid sequence operably linked to a promoter sequence. Such a recombinant nucleic acid may be part of a vector that is used, for example, to transform a cell.

Alternatively, such recombinant nucleic acids may be part of a viral vector, e.g., based on a  
20 vaccinia virus, that could be used to vaccinate a mammal wherein the recombinant nucleic acid is expressed, inducing a protective immunological response in the mammal.

A "regulatory element" refers to a nucleic acid sequence usually derived from untranslated regions of a gene and includes enhancers, promoters, introns, and 5' and 3' untranslated regions (UTRs). Regulatory elements interact with host or viral proteins which control transcription,  
25 translation, or RNA stability.

"Reporter molecules" are chemical or biochemical moieties used for labeling a nucleic acid, amino acid, or antibody. Reporter molecules include radionuclides; enzymes; fluorescent, chemiluminescent, or chromogenic agents; substrates; cofactors; inhibitors; magnetic particles; and other moieties known in the art.

30 An "RNA equivalent," in reference to a DNA sequence, is composed of the same linear sequence of nucleotides as the reference DNA sequence with the exception that all occurrences of the nitrogenous base thymine are replaced with uracil, and the sugar backbone is composed of ribose instead of deoxyribose.

The term "sample" is used in its broadest sense. A sample suspected of containing nucleic  
35 acids encoding SYNT, or fragments thereof, or SYNT itself, may comprise a bodily fluid; an extract from a cell, chromosome, organelle, or membrane isolated from a cell; a cell; genomic DNA, RNA, or



cDNA, in solution or bound to a substrate; a tissue; a tissue print; etc.

The terms "specific binding" and "specifically binding" refer to that interaction between a protein or peptide and an agonist, an antibody, an antagonist, a small molecule, or any natural or synthetic binding composition. The interaction is dependent upon the presence of a particular structure of the protein, e.g., the antigenic determinant or epitope, recognized by the binding molecule. For example, if an antibody is specific for epitope "A," the presence of a polypeptide comprising the epitope A, or the presence of free unlabeled A, in a reaction containing free labeled A and the antibody will reduce the amount of labeled A that binds to the antibody.

The term "substantially purified" refers to nucleic acid or amino acid sequences that are removed from their natural environment and are isolated or separated, and are at least 60% free, preferably at least 75% free, and most preferably at least 90% free from other components with which they are naturally associated.

A "substitution" refers to the replacement of one or more amino acid residues or nucleotides by different amino acid residues or nucleotides, respectively.

"Substrate" refers to any suitable rigid or semi-rigid support including membranes, filters, chips, slides, wafers, fibers, magnetic or nonmagnetic beads, gels, tubing, plates, polymers, microparticles and capillaries. The substrate can have a variety of surface forms, such as wells, trenches, pins, channels and pores, to which polynucleotides or polypeptides are bound.

A "transcript image" refers to the collective pattern of gene expression by a particular cell type or tissue under given conditions at a given time.

"Transformation" describes a process by which exogenous DNA is introduced into a recipient cell. Transformation may occur under natural or artificial conditions according to various methods well known in the art, and may rely on any known method for the insertion of foreign nucleic acid sequences into a prokaryotic or eukaryotic host cell. The method for transformation is selected based on the type of host cell being transformed and may include, but is not limited to, bacteriophage or viral infection, electroporation, heat shock, lipofection, and particle bombardment. The term "transformed" cells includes stably transformed cells in which the inserted DNA is capable of replication either as an autonomously replicating plasmid or as part of the host chromosome, as well as transiently transformed cells which express the inserted DNA or RNA for limited periods of time.

A "transgenic organism," as used herein, is any organism, including but not limited to animals and plants, in which one or more of the cells of the organism contains heterologous nucleic acid introduced by way of human intervention, such as by transgenic techniques well known in the art. The nucleic acid is introduced into the cell, directly or indirectly by introduction into a precursor of the cell, by way of deliberate genetic manipulation, such as by microinjection or by infection with a recombinant virus. The term genetic manipulation does not include classical cross-breeding, or in vitro fertilization, but rather is directed to the introduction of a recombinant DNA molecule. The

transgenic organisms contemplated in accordance with the present invention include bacteria, cyanobacteria, fungi, plants, and animals. The isolated DNA of the present invention can be introduced into the host by methods known in the art, for example infection, transfection, transformation or transconjugation. Techniques for transferring the DNA of the present invention into such organisms are widely known and provided in references such as Sambrook et al. (1989), supra.

A "variant" of a particular nucleic acid sequence is defined as a nucleic acid sequence having at least 40% sequence identity to the particular nucleic acid sequence over a certain length of one of the nucleic acid sequences using blastn with the "BLAST 2 Sequences" tool Version 2.0.9 (May-07-1999) set at default parameters. Such a pair of nucleic acids may show, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 95% or at least 98% or greater sequence identity over a certain defined length. A variant may be described as, for example, an "allelic" (as defined above), "splice," "species," or "polymorphic" variant. A splice variant may have significant identity to a reference molecule, but will generally have a greater or lesser number of polynucleotides due to alternative splicing of exons during mRNA processing. The corresponding polypeptide may possess additional functional domains or lack domains that are present in the reference molecule. Species variants are polynucleotide sequences that vary from one species to another. The resulting polypeptides generally will have significant amino acid identity relative to each other. A polymorphic variant is a variation in the polynucleotide sequence of a particular gene between individuals of a given species. Polymorphic variants also may encompass "single nucleotide polymorphisms" (SNPs) in which the polynucleotide sequence varies by one nucleotide base. The presence of SNPs may be indicative of, for example, a certain population, a disease state, or a propensity for a disease state.

A "variant" of a particular polypeptide sequence is defined as a polypeptide sequence having at least 40% sequence identity to the particular polypeptide sequence over a certain length of one of the polypeptide sequences using blastp with the "BLAST 2 Sequences" tool Version 2.0.9 (May-07-1999) set at default parameters. Such a pair of polypeptides may show, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 98% or greater sequence identity over a certain defined length of one of the polypeptides.

### 30 THE INVENTION

The invention is based on the discovery of new human synthetases (SYNT), the polynucleotides encoding SYNT, and the use of these compositions for the diagnosis, treatment, or prevention of immune, neuronal, and reproductive disorders, and cell proliferative disorders including cancer.

35 Table 1 lists the Incyte clones used to assemble full length nucleotide sequences encoding SYNT. Columns 1 and 2 show the sequence identification numbers (SEQ ID NOs) of the polypeptide

and nucleotide sequences, respectively. Column 3 shows the clone IDs of the Incyte clones in which nucleic acids encoding each SYNT were identified, and column 4 shows the cDNA libraries from which these clones were isolated. Column 5 shows Incyte clones and their corresponding cDNA libraries. Clones for which cDNA libraries are not indicated were derived from pooled cDNA libraries. In some cases, GenBank sequence identifiers are also shown in column 5. The Incyte clones and GenBank cDNA sequences, where indicated, in column 5 were used to assemble the consensus nucleotide sequence of each SYNT and are useful as fragments in hybridization technologies.

The columns of Table 2 show various properties of each of the polypeptides of the invention: column 1 references the SEQ ID NO; column 2 shows the number of amino acid residues in each polypeptide; column 3 shows potential phosphorylation sites; column 4 shows potential glycosylation sites; column 5 shows the amino acid residues comprising signature sequences and motifs; column 6 shows homologous sequences as identified by BLAST analysis; and column 7 shows analytical methods and in some cases, searchable databases to which the analytical methods were applied. The methods of column 7 were used to characterize each polypeptide through sequence homology and protein motifs.

The columns of Table 3 show the tissue-specificity and diseases, disorders, or conditions associated with nucleotide sequences encoding SYNT. The first column of Table 3 lists the nucleotide SEQ ID NOs. Column 2 lists fragments of the nucleotide sequences of column 1. These fragments are useful, for example, in hybridization or amplification technologies to identify SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:29, and SEQ ID NO:30 and to distinguish between SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:29, and SEQ ID NO:30 and related polynucleotide sequences. The polypeptides encoded by these fragments are useful, for example, as immunogenic peptides. Column 3 lists tissue categories which express SYNT as a fraction of total tissues expressing SYNT. Column 4 lists diseases, disorders, or conditions associated with those tissues expressing SYNT as a fraction of total tissues expressing SYNT. Column 5 lists the vectors used to subclone each cDNA library.

The columns of Table 4 show descriptions of the tissues used to construct the cDNA libraries from which cDNA clones encoding SYNT were isolated. Column 1 references the nucleotide SEQ ID NOs, column 2 shows the cDNA libraries from which these clones were isolated, and column 3 shows the tissue origins and other descriptive information relevant to the cDNA libraries in column 2.

SEQ ID NO: 16 maps to chromosome 5 within the interval from 147.10 to 150.00 centiMorgans. SEQ ID NO: 17 maps to chromosome 10 within the interval from 137.60 to 139.20 centiMorgans. This interval also contains gene MXI1, a member of the MYC family. SEQ ID NO:

18 maps to chromosome 2 within the interval from 228.80 to 230.10 centiMorgans. This interval also contains a gene for a proto-oncogene encoding a tyrosine-protein kinase. SEQ ID NO:21 maps to chromosome 5 within the interval from 172.6 to 184.7 centiMorgans. SEQ ID NO:24 maps to chromosome 2 within the interval from 118.0 to 127.4 centiMorgans. SEQ ID NO:26 maps to  
5 chromosome 3 within the interval from 157.4 to 162.0 centiMorgans. SEQ ID NO:27 maps to chromosome 12 within the interval from 97.1 to 116.6 centiMorgans. SEQ ID NO:28 maps to chromosome 4 within the interval from 77.3 to 99.2 centiMorgans and to chromosome 5 within the intervals from 79.2 to 92.3 centiMorgans, from 116.3 to 127.9 centiMorgans, and from 157.6 to 163.0 centiMorgans. SEQ ID NO:29 maps to chromosome 1 within the interval from 242.5 to 258.7  
10 centiMorgans and to chromosome 19 within the interval from 69.9 to 104.9 centiMorgans. SEQ ID NO:30 maps to chromosome 1 within the interval from 57.2 to 57.5 centiMorgans.

The invention also encompasses SYNT variants. A preferred SYNT variant is one which has at least about 80%, or alternatively at least about 90%, or even at least about 95% amino acid sequence identity to the SYNT amino acid sequence, and which contains at least one functional or  
15 structural characteristic of SYNT.

The invention also encompasses polynucleotides which encode SYNT. In a particular embodiment, the invention encompasses a polynucleotide sequence comprising a sequence selected from the group consisting of SEQ ID NO:16-30, which encodes SYNT. The polynucleotide sequences of SEQ ID NO:16-30, as presented in the Sequence Listing, embrace the equivalent RNA  
20 sequences, wherein occurrences of the nitrogenous base thymine are replaced with uracil, and the sugar backbone is composed of ribose instead of deoxyribose.

The invention also encompasses a variant of a polynucleotide sequence encoding SYNT. In particular, such a variant polynucleotide sequence will have at least about 70%, or alternatively at least about 85%, or even at least about 95% polynucleotide sequence identity to the polynucleotide  
25 sequence encoding SYNT. A particular aspect of the invention encompasses a variant of a polynucleotide sequence comprising a sequence selected from the group consisting of SEQ ID NO:16-30 which has at least about 70%, or alternatively at least about 85%, or even at least about 95% polynucleotide sequence identity to a nucleic acid sequence selected from the group consisting of SEQ ID NO:16-30. Any one of the polynucleotide variants described above can encode an amino  
30 acid sequence which contains at least one functional or structural characteristic of SYNT.

It will be appreciated by those skilled in the art that as a result of the degeneracy of the genetic code, a multitude of polynucleotide sequences encoding SYNT, some bearing minimal similarity to the polynucleotide sequences of any known and naturally occurring gene, may be produced. Thus, the invention contemplates each and every possible variation of polynucleotide  
35 sequence that could be made by selecting combinations based on possible codon choices. These combinations are made in accordance with the standard triplet genetic code as applied to the

polynucleotide sequence of naturally occurring SYNT, and all such variations are to be considered as being specifically disclosed.

Although nucleotide sequences which encode SYNT and its variants are generally capable of hybridizing to the nucleotide sequence of the naturally occurring SYNT under appropriately selected conditions of stringency, it may be advantageous to produce nucleotide sequences encoding SYNT or its derivatives possessing a substantially different codon usage, e.g., inclusion of non-naturally occurring codons. Codons may be selected to increase the rate at which expression of the peptide occurs in a particular prokaryotic or eukaryotic host in accordance with the frequency with which particular codons are utilized by the host. Other reasons for substantially altering the nucleotide sequence encoding SYNT and its derivatives without altering the encoded amino acid sequences include the production of RNA transcripts having more desirable properties, such as a greater half-life, than transcripts produced from the naturally occurring sequence.

The invention also encompasses production of DNA sequences which encode SYNT and SYNT derivatives, or fragments thereof, entirely by synthetic chemistry. After production, the synthetic sequence may be inserted into any of the many available expression vectors and cell systems using reagents well known in the art. Moreover, synthetic chemistry may be used to introduce mutations into a sequence encoding SYNT or any fragment thereof.

Also encompassed by the invention are polynucleotide sequences that are capable of hybridizing to the claimed polynucleotide sequences, and, in particular, to those shown in SEQ ID NO:16-30 and fragments thereof under various conditions of stringency. (See, e.g., Wahl, G.M. and S.L. Berger (1987) *Methods Enzymol.* 152:399-407; Kimmel, A.R. (1987) *Methods Enzymol.* 152:507-511.) Hybridization conditions, including annealing and wash conditions, are described in "Definitions."

Methods for DNA sequencing are well known in the art and may be used to practice any of the embodiments of the invention. The methods may employ such enzymes as the Klenow fragment of DNA polymerase I, SEQUENASE (US Biochemical, Cleveland OH), Taq polymerase (PE Biosystems, Foster City CA), thermostable T7 polymerase (Amersham Pharmacia Biotech, Piscataway NJ), or combinations of polymerases and proofreading exonucleases such as those found in the ELONGASE amplification system (Life Technologies, Gaithersburg MD). Preferably, sequence preparation is automated with machines such as the MICROLAB 2200 liquid transfer system (Hamilton, Reno NV), PTC200 thermal cycler (MJ Research, Watertown MA) and ABI CATALYST 800 thermal cycler (PE Biosystems). Sequencing is then carried out using either the ABI 373 or 377 DNA sequencing system (PE Biosystems), the MEGABACE 1000 DNA sequencing system (Molecular Dynamics, Sunnyvale CA), or other systems known in the art. The resulting sequences are analyzed using a variety of algorithms which are well known in the art. (See, e.g., Ausubel, F.M. (1997) Short Protocols in Molecular Biology, John Wiley & Sons, New York NY, unit

7.7: Meyers, R.A. (1995) Molecular Biology and Biotechnology, Wiley VCH, New York NY, pp. 856-853.)

The nucleic acid sequences encoding SYNT may be extended utilizing a partial nucleotide sequence and employing various PCR-based methods known in the art to detect upstream sequences, such as promoters and regulatory elements. For example, one method which may be employed, restriction-site PCR, uses universal and nested primers to amplify unknown sequence from genomic DNA within a cloning vector. (See, e.g., Sarkar, G. (1993) PCR Methods Applic. 2:318-322.) Another method, inverse PCR, uses primers that extend in divergent directions to amplify unknown sequence from a circularized template. The template is derived from restriction fragments comprising a known genomic locus and surrounding sequences. (See, e.g., Triglia, T. et al. (1988) Nucleic Acids Res. 16:8186.) A third method, capture PCR, involves PCR amplification of DNA fragments adjacent to known sequences in human and yeast artificial chromosome DNA. (See, e.g., Lagerstrom, M. et al. (1991) PCR Methods Applic. 1:111-119.) In this method, multiple restriction enzyme digestions and ligations may be used to insert an engineered double-stranded sequence into a region of unknown sequence before performing PCR. Other methods which may be used to retrieve unknown sequences are known in the art. (See, e.g., Parker, J.D. et al. (1991) Nucleic Acids Res. 19:3055-3060). Additionally, one may use PCR, nested primers, and PROMOTERFINDER libraries (Clontech, Palo Alto CA) to walk genomic DNA. This procedure avoids the need to screen libraries and is useful in finding intron/exon junctions. For all PCR-based methods, primers may be designed using commercially available software, such as OLIGO 4.06 Primer Analysis software (National Biosciences, Plymouth MN) or another appropriate program, to be about 22 to 30 nucleotides in length, to have a GC content of about 50% or more, and to anneal to the template at temperatures of about 68°C to 72°C.

When screening for full-length cDNAs, it is preferable to use libraries that have been size-selected to include larger cDNAs. In addition, random-primed libraries, which often include sequences containing the 5' regions of genes, are preferable for situations in which an oligo d(T) library does not yield a full-length cDNA. Genomic libraries may be useful for extension of sequence into 5' non-transcribed regulatory regions.

Capillary electrophoresis systems which are commercially available may be used to analyze the size or confirm the nucleotide sequence of sequencing or PCR products. In particular, capillary sequencing may employ flowable polymers for electrophoretic separation, four different nucleotide-specific, laser-stimulated fluorescent dyes, and a charge coupled device camera for detection of the emitted wavelengths. Output/light intensity may be converted to electrical signal using appropriate software (e.g., GENOTYPER and SEQUENCE NAVIGATOR, PE Biosystems), and the entire process from loading of samples to computer analysis and electronic data display may be computer controlled. Capillary electrophoresis is especially preferable for sequencing small DNA fragments

which may be present in limited amounts in a particular sample.

In another embodiment of the invention, polynucleotide sequences or fragments thereof which encode SYNT may be cloned in recombinant DNA molecules that direct expression of SYNT, or fragments or functional equivalents thereof, in appropriate host cells. Due to the inherent  
5 degeneracy of the genetic code, other DNA sequences which encode substantially the same or a functionally equivalent amino acid sequence may be produced and used to express SYNT.

The nucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter SYNT-encoding sequences for a variety of purposes including, but not limited to, modification of the cloning, processing, and/or expression of the gene product. DNA  
10 shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. For example, oligonucleotide-mediated site-directed mutagenesis may be used to introduce mutations that create new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, and so forth.

The nucleotides of the present invention may be subjected to DNA shuffling techniques such  
15 as MOLECULARBREEDING (Maxygen Inc., Santa Clara CA; described in U.S. Patent Number 5,837,458; Chang, C.-C. et al. (1999) *Nat. Biotechnol.* 17:793-797; Christians, F.C. et al. (1999) *Nat. Biotechnol.* 17:259-264; and Cramer, A. et al. (1996) *Nat. Biotechnol.* 14:315-319) to alter or improve the biological properties of SYNT, such as its biological or enzymatic activity or its ability to bind to other molecules or compounds. DNA shuffling is a process by which a library of gene  
20 variants is produced using PCR-mediated recombination of gene fragments. The library is then subjected to selection or screening procedures that identify those gene variants with the desired properties. These preferred variants may then be pooled and further subjected to recursive rounds of DNA shuffling and selection/screening. Thus, genetic diversity is created through "artificial" breeding and rapid molecular evolution. For example, fragments of a single gene containing random  
25 point mutations may be recombined, screened, and then reshuffled until the desired properties are optimized. Alternatively, fragments of a given gene may be recombined with fragments of homologous genes in the same gene family, either from the same or different species, thereby maximizing the genetic diversity of multiple naturally occurring genes in a directed and controllable manner.

30 In another embodiment, sequences encoding SYNT may be synthesized, in whole or in part, using chemical methods well known in the art. (See, e.g., Caruthers, M.H. et al. (1980) *Nucleic Acids Symp. Ser.* 7:215-223; and Horn, T. et al. (1980) *Nucleic Acids Symp. Ser.* 7:225-232.) Alternatively, SYNT itself or a fragment thereof may be synthesized using chemical methods. For example, peptide synthesis can be performed using various solution-phase or solid-phase techniques.  
35 (See, e.g., Creighton, T. (1984) Proteins, Structures and Molecular Properties, WH Freeman, New York NY, pp. 55-60; and Roberge, J.Y. et al. (1995) *Science* 269:202-204.) Automated synthesis

may be achieved using the ABI 431A peptide synthesizer (PE Biosystems). Additionally, the amino acid sequence of SYNT, or any part thereof, may be altered during direct synthesis and/or combined with sequences from other proteins, or any part thereof, to produce a variant polypeptide or a polypeptide having a sequence of a naturally occurring polypeptide.

5       The peptide may be substantially purified by preparative high performance liquid chromatography. (See, e.g., Chiez, R.M. and F.Z. Regnier (1990) *Methods Enzymol.* 182:392-421.) The composition of the synthetic peptides may be confirmed by amino acid analysis or by sequencing. (See, e.g., Creighton, supra, pp. 28-53.)

10       In order to express a biologically active SYNT, the nucleotide sequences encoding SYNT or derivatives thereof may be inserted into an appropriate expression vector, i.e., a vector which contains the necessary elements for transcriptional and translational control of the inserted coding sequence in a suitable host. These elements include regulatory sequences, such as enhancers, constitutive and inducible promoters, and 5' and 3' untranslated regions in the vector and in polynucleotide sequences encoding SYNT. Such elements may vary in their strength and specificity. Specific initiation signals  
15       may also be used to achieve more efficient translation of sequences encoding SYNT. Such signals include the ATG initiation codon and adjacent sequences, e.g. the Kozak sequence. In cases where sequences encoding SYNT and its initiation codon and upstream regulatory sequences are inserted into the appropriate expression vector, no additional transcriptional or translational control signals may be needed. However, in cases where only coding sequence, or a fragment thereof, is inserted,  
20       exogenous translational control signals including an in-frame ATG initiation codon should be provided by the vector. Exogenous translational elements and initiation codons may be of various origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of enhancers appropriate for the particular host cell system used. (See, e.g., Scharf, D. et al. (1994) *Results Probl. Cell Differ.* 20:125-162.)

25       Methods which are well known to those skilled in the art may be used to construct expression vectors containing sequences encoding SYNT and appropriate transcriptional and translational control elements. These methods include in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. (See, e.g., Sambrook, J. et al. (1989) Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press, Plainview NY, ch. 4, 8, and 16-17; Ausubel, F.M. et  
30       al. (1995) Current Protocols in Molecular Biology, John Wiley & Sons, New York NY, ch. 9, 13, and 16.)

35       A variety of expression vector/host systems may be utilized to contain and express sequences encoding SYNT. These include, but are not limited to, microorganisms such as bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA expression vectors; yeast transformed with yeast expression vectors; insect cell systems infected with viral expression vectors (e.g., baculovirus); plant cell systems transformed with viral expression vectors (e.g., cauliflower mosaic virus, CaMV,



or tobacco mosaic virus, TMV) or with bacterial expression vectors (e.g., Ti or pBR322 plasmids); or animal cell systems. (See, e.g., Sambrook, supra; Ausubel, supra; Van Heeke, G. and S.M. Schuster (1989) J. Biol. Chem. 264:5503-5509; Bitter, G.A. et al. (1987) Methods Enzymol. 153:516-544; Scorer, C.A. et al. (1994) Bio/Technology 12:181-184; Engelhard, E.K. et al. (1994) Proc. Natl. Acad. Sci. USA 91:3224-3227; Sandig, V. et al. (1996) Hum. Gene Ther. 7:1937-1945; Takamatsu, N. (1987) EMBO J. 6:307-311; Coruzzi, G. et al. (1984) EMBO J. 3:1671-1680; Broglie, R. et al. (1984) Science 224:838-843; Winter, J. et al. (1991) Results Probl. Cell Differ. 17:85-105; The McGraw Hill Yearbook of Science and Technology (1992) McGraw Hill, New York NY, pp. 191-196; Logan, J. and T. Shenk (1984) Proc. Natl. Acad. Sci. USA 81:3655-3659; and Harrington, J.J. et al. (1997) Nat. Genet. 15:345-355.) Expression vectors derived from retroviruses, adenoviruses, or herpes or vaccinia viruses, or from various bacterial plasmids, may be used for delivery of nucleotide sequences to the targeted organ, tissue, or cell population. (See, e.g., Di Nicola, M. et al. (1998) Cancer Gen. Ther. 5(6):350-356; Yu, M. et al., (1993) Proc. Natl. Acad. Sci. USA 90(13):6340-6344; Buller, R.M. et al. (1985) Nature 317(6040):813-815; McGregor, D.P. et al. (1994) Mol. Immunol. 31(3):219-226; and Verma, I.M. and N. Somia (1997) Nature 389:239-242.) The invention is not limited by the host cell employed.

In bacterial systems, a number of cloning and expression vectors may be selected depending upon the use intended for polynucleotide sequences encoding SYNT. For example, routine cloning, subcloning, and propagation of polynucleotide sequences encoding SYNT can be achieved using a multifunctional *E. coli* vector such as PBLUESCRIPT (Stratagene, La Jolla CA) or PSPORT1 plasmid (Life Technologies). Ligation of sequences encoding SYNT into the vector's multiple cloning site disrupts the *lacZ* gene, allowing a colorimetric screening procedure for identification of transformed bacteria containing recombinant molecules. In addition, these vectors may be useful for *in vitro* transcription, dideoxy sequencing, single strand rescue with helper phage, and creation of nested deletions in the cloned sequence. (See, e.g., Van Heeke, G. and S.M. Schuster (1989) J. Biol. Chem. 264:5503-5509.) When large quantities of SYNT are needed, e.g. for the production of antibodies, vectors which direct high level expression of SYNT may be used. For example, vectors containing the strong, inducible T5 or T7 bacteriophage promoter may be used.

Yeast expression systems may be used for production of SYNT. A number of vectors containing constitutive or inducible promoters, such as alpha factor, alcohol oxidase, and PGH promoters, may be used in the yeast *Saccharomyces cerevisiae* or *Pichia pastoris*. In addition, such vectors direct either the secretion or intracellular retention of expressed proteins and enable integration of foreign sequences into the host genome for stable propagation. (See, e.g., Ausubel, 1995, supra; Bitter, supra; and Scorer, supra.)

Plant systems may also be used for expression of SYNT. Transcription of sequences encoding SYNT may be driven viral promoters, e.g., the 35S and 19S promoters of CaMV used alone

or in combination with the omega leader sequence from TMV (Takamatsu, N. (1987) EMBO J. 6:307-311). Alternatively, plant promoters such as the small subunit of RUBISCO or heat shock promoters may be used. (See, e.g., Coruzzi, supra; Broglie, supra; and Winter, supra.) These constructs can be introduced into plant cells by direct DNA transformation or pathogen-mediated transfection. (See, e.g., The McGraw Hill Yearbook of Science and Technology (1992) McGraw Hill, New York NY, pp. 191-196.)

In mammalian cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, sequences encoding SYNT may be ligated into an adenovirus transcription/translation complex consisting of the late promoter and tripartite leader sequence. Insertion in a non-essential E1 or E3 region of the viral genome may be used to obtain infective virus which expresses SYNT in host cells. (See, e.g., Logan, J. and T. Shenk (1984) Proc. Natl. Acad. Sci. USA 81:3655-3659.) In addition, transcription enhancers, such as the Rous sarcoma virus (RSV) enhancer, may be used to increase expression in mammalian host cells. SV40 or EBV-based vectors may also be used for high-level protein expression.

Human artificial chromosomes (HACs) may also be employed to deliver larger fragments of DNA than can be contained in and expressed from a plasmid. HACs of about 6 kb to 10 Mb are constructed and delivered via conventional delivery methods (liposomes, polycationic amino polymers, or vesicles) for therapeutic purposes. (See, e.g., Harrington, J.J. et al. (1997) Nat. Genet. 15:345-355.)

For long term production of recombinant proteins in mammalian systems, stable expression of SYNT in cell lines is preferred. For example, sequences encoding SYNT can be transformed into cell lines using expression vectors which may contain viral origins of replication and/or endogenous expression elements and a selectable marker gene on the same or on a separate vector. Following the introduction of the vector, cells may be allowed to grow for about 1 to 2 days in enriched media before being switched to selective media. The purpose of the selectable marker is to confer resistance to a selective agent, and its presence allows growth and recovery of cells which successfully express the introduced sequences. Resistant clones of stably transformed cells may be propagated using tissue culture techniques appropriate to the cell type.

Any number of selection systems may be used to recover transformed cell lines. These include, but are not limited to, the herpes simplex virus thymidine kinase and adenine phosphoribosyltransferase genes, for use in *tk<sup>-</sup>* and *apr<sup>-</sup>* cells, respectively. (See, e.g., Wigler, M. et al. (1977) Cell 11:223-232; Lowy, I. et al. (1980) Cell 22:817-823.) Also, antimetabolite, antibiotic, or herbicide resistance can be used as the basis for selection. For example, *dhfr* confers resistance to methotrexate; *neo* confers resistance to the aminoglycosides neomycin and G-418; and *als* and *pat* confer resistance to chlorsulfuron and phosphinotricin acetyltransferase, respectively. (See, e.g., Wigler, M. et al. (1980) Proc. Natl. Acad. Sci. USA 77:3567-3570; Colbere-Garapin, F. et al. (1981)

J. Mol. Biol. 150:1-14.) Additional selectable genes have been described, e.g., *trpB* and *hisD*, which alter cellular requirements for metabolites. (See, e.g., Hartman, S.C. and R.C. Mulligan (1988) Proc. Natl. Acad. Sci. USA 85:8047-8051.) Visible markers, e.g., anthocyanins, green fluorescent proteins (GFP; Clontech),  $\beta$  glucuronidase and its substrate  $\beta$ -glucuronide, or luciferase and its substrate luciferin may be used. These markers can be used not only to identify transformants, but also to quantify the amount of transient or stable protein expression attributable to a specific vector system. (See, e.g., Rhodes, C.A. (1995) Methods Mol. Biol. 55:121-131.)

Although the presence/absence of marker gene expression suggests that the gene of interest is also present, the presence and expression of the gene may need to be confirmed. For example, if the sequence encoding SYNT is inserted within a marker gene sequence, transformed cells containing sequences encoding SYNT can be identified by the absence of marker gene function. Alternatively, a marker gene can be placed in tandem with a sequence encoding SYNT under the control of a single promoter. Expression of the marker gene in response to induction or selection usually indicates expression of the tandem gene as well.

In general, host cells that contain the nucleic acid sequence encoding SYNT and that express SYNT may be identified by a variety of procedures known to those of skill in the art. These procedures include, but are not limited to, DNA-DNA or DNA-RNA hybridizations, PCR amplification, and protein bioassay or immunoassay techniques which include membrane, solution, or chip based technologies for the detection and/or quantification of nucleic acid or protein sequences.

Immunological methods for detecting and measuring the expression of SYNT using either specific polyclonal or monoclonal antibodies are known in the art. Examples of such techniques include enzyme-linked immunosorbent assays (ELISAs), radioimmunoassays (RIAs), and fluorescence activated cell sorting (FACS). A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes on SYNT is preferred, but a competitive binding assay may be employed. These and other assays are well known in the art. (See, e.g., Hampton, R. et al. (1990) Serological Methods, a Laboratory Manual, APS Press, St. Paul MN, Sect. IV; Coligan, J.E. et al. (1997) Current Protocols in Immunology, Greene Pub. Associates and Wiley-Interscience, New York NY; and Pound, J.D. (1998) Immunochemical Protocols, Humana Press, Totowa NJ.)

A wide variety of labels and conjugation techniques are known by those skilled in the art and may be used in various nucleic acid and amino acid assays. Means for producing labeled hybridization or PCR probes for detecting sequences related to polynucleotides encoding SYNT include oligolabeling, nick translation, end-labeling, or PCR amplification using a labeled nucleotide. Alternatively, the sequences encoding SYNT, or any fragments thereof, may be cloned into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes *in vitro* by addition of an appropriate RNA polymerase

such as T7, T3, or SP6 and labeled nucleotides. These procedures may be conducted using a variety of commercially available kits, such as those provided by Amersham Pharmacia Biotech, Promega (Madison WI), and US Biochemical. Suitable reporter molecules or labels which may be used for ease of detection include radionuclides, enzymes, fluorescent, chemiluminescent, or chromogenic agents, as well as substrates, cofactors, inhibitors, magnetic particles, and the like.

Host cells transformed with nucleotide sequences encoding SYNT may be cultured under conditions suitable for the expression and recovery of the protein from cell culture. The protein produced by a transformed cell may be secreted or retained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing polynucleotides which encode SYNT may be designed to contain signal sequences which direct secretion of SYNT through a prokaryotic or eukaryotic cell membrane.

In addition, a host cell strain may be chosen for its ability to modulate expression of the inserted sequences or to process the expressed protein in the desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation, and acylation. Post-translational processing which cleaves a "prepro" or "pro" form of the protein may also be used to specify protein targeting, folding, and/or activity. Different host cells which have specific cellular machinery and characteristic mechanisms for post-translational activities (e.g., CHO, HeLa, MDCK, HEK293, and WI38) are available from the American Type Culture Collection (ATCC, Manassas VA) and may be chosen to ensure the correct modification and processing of the foreign protein.

In another embodiment of the invention, natural, modified, or recombinant nucleic acid sequences encoding SYNT may be ligated to a heterologous sequence resulting in translation of a fusion protein in any of the aforementioned host systems. For example, a chimeric SYNT protein containing a heterologous moiety that can be recognized by a commercially available antibody may facilitate the screening of peptide libraries for inhibitors of SYNT activity. Heterologous protein and peptide moieties may also facilitate purification of fusion proteins using commercially available affinity matrices. Such moieties include, but are not limited to, glutathione S-transferase (GST), maltose binding protein (MBP), thioredoxin (Trx), calmodulin binding peptide (CBP), 6-His, FLAG, *c-myc*, and hemagglutinin (HA). GST, MBP, Trx, CBP, and 6-His enable purification of their cognate fusion proteins on immobilized glutathione, maltose, phenylarsine oxide, calmodulin, and metal-chelate resins, respectively. FLAG, *c-myc*, and hemagglutinin (HA) enable immunoaffinity purification of fusion proteins using commercially available monoclonal and polyclonal antibodies that specifically recognize these epitope tags. A fusion protein may also be engineered to contain a proteolytic cleavage site located between the SYNT encoding sequence and the heterologous protein sequence, so that SYNT may be cleaved away from the heterologous moiety following purification. Methods for fusion protein expression and purification are discussed in Ausubel (1995, supra, ch. 10).

A variety of commercially available kits may also be used to facilitate expression and purification of fusion proteins.

In a further embodiment of the invention, synthesis of radiolabeled SYNT may be achieved in vitro using the TNT rabbit reticulocyte lysate or wheat germ extract system (Promega). These systems couple transcription and translation of protein-coding sequences operably associated with the T7, T3, or SP6 promoters. Translation takes place in the presence of a radiolabeled amino acid precursor, for example, <sup>35</sup>S-methionine.

SYNT of the present invention or fragments thereof may be used to screen for compounds that specifically bind to SYNT. At least one and up to a plurality of test compounds may be screened for specific binding to SYNT. Examples of test compounds include antibodies, oligonucleotides, proteins (e.g., receptors), or small molecules.

In one embodiment, the compound thus identified is closely related to the natural ligand of SYNT, e.g., a ligand or fragment thereof, a natural substrate, a structural or functional mimetic, or a natural binding partner. (See, Coligan, J.E. et al. (1991) Current Protocols in Immunology 1(2): Chapter 5.) Similarly, the compound can be closely related to the natural receptor to which SYNT binds, or to at least a fragment of the receptor, e.g., the ligand binding site. In either case, the compound can be rationally designed using known techniques. In one embodiment, screening for these compounds involves producing appropriate cells which express SYNT, either as a secreted protein or on the cell membrane. Preferred cells include cells from mammals, yeast, Drosophila, or E. coli. Cells expressing SYNT or cell membrane fractions which contain SYNT are then contacted with a test compound and binding, stimulation, or inhibition of activity of either SYNT or the compound is analyzed.

An assay may simply test binding of a test compound to the polypeptide, wherein binding is detected by a fluorophore, radioisotope, enzyme conjugate, or other detectable label. For example, the assay may comprise the steps of combining at least one test compound with SYNT, either in solution or affixed to a solid support, and detecting the binding of SYNT to the compound. Alternatively, the assay may detect or measure binding of a test compound in the presence of a labeled competitor. Additionally, the assay may be carried out using cell-free preparations, chemical libraries, or natural product mixtures, and the test compound(s) may be free in solution or affixed to a solid support.

SYNT of the present invention or fragments thereof may be used to screen for compounds that modulate the activity of SYNT. Such compounds may include agonists, antagonists, or partial or inverse agonists. In one embodiment, an assay is performed under conditions permissive for SYNT activity, wherein SYNT is combined with at least one test compound, and the activity of SYNT in the presence of a test compound is compared with the activity of SYNT in the absence of the test compound. A change in the activity of SYNT in the presence of the test compound is indicative of a

compound that modulates the activity of SYNT. Alternatively, a test compound is combined with an in vitro or cell-free system comprising SYNT under conditions suitable for SYNT activity, and the assay is performed. In either of these assays, a test compound which modulates the activity of SYNT may do so indirectly and need not come in direct contact with the test compound. At least one and up to a plurality of test compounds may be screened.

In another embodiment, polynucleotides encoding SYNT or their mammalian homologs may be "knocked out" in an animal model system using homologous recombination in embryonic stem (ES) cells. Such techniques are well known in the art and are useful for the generation of animal models of human disease. (See, e.g., U.S. Patent No. 5,175,383 and U.S. Patent No. 5,767,337.) For example, mouse ES cells, such as the mouse 129/SvJ cell line, are derived from the early mouse embryo and grown in culture. The ES cells are transformed with a vector containing the gene of interest disrupted by a marker gene, e.g., the neomycin phosphotransferase gene (neo; Capecchi, M.R. (1989) Science 244:1288-1292). The vector integrates into the corresponding region of the host genome by homologous recombination. Alternatively, homologous recombination takes place using the Cre-loxP system to knockout a gene of interest in a tissue- or developmental stage-specific manner (Marth, J.D. (1996) Clin. Invest. 97:1999-2002; Wagner, K.U. et al. (1997) Nucleic Acids Res. 25:4323-4330). Transformed ES cells are identified and microinjected into mouse cell blastocysts such as those from the C57BL/6 mouse strain. The blastocysts are surgically transferred to pseudopregnant dams, and the resulting chimeric progeny are genotyped and bred to produce heterozygous or homozygous strains. Transgenic animals thus generated may be tested with potential therapeutic or toxic agents.

Polynucleotides encoding SYNT may also be manipulated in vitro in ES cells derived from human blastocysts. Human ES cells have the potential to differentiate into at least eight separate cell lineages including endoderm, mesoderm, and ectodermal cell types. These cell lineages differentiate into, for example, neural cells, hematopoietic lineages, and cardiomyocytes (Thomson, J.A. et al. (1998) Science 282:1145-1147).

Polynucleotides encoding SYNT can also be used to create "knockin" humanized animals (pigs) or transgenic animals (mice or rats) to model human disease. With knockin technology, a region of a polynucleotide encoding SYNT is injected into animal ES cells, and the injected sequence integrates into the animal cell genome. Transformed cells are injected into blastulae, and the blastulae are implanted as described above. Transgenic progeny or inbred lines are studied and treated with potential pharmaceutical agents to obtain information on treatment of a human disease. Alternatively, a mammal inbred to overexpress SYNT, e.g., by secreting SYNT in its milk, may also serve as a convenient source of that protein (Janne, J. et al. (1998) Biotechnol. Annu. Rev. 4:55-74).

## **THERAPEUTICS**

Chemical and structural similarity, e.g., in the context of sequences and motifs, exists

between regions of SYNT and human synthetases. In addition, the expression of SYNT is closely associated with hematopoietic/immune, cancerous, proliferating, inflamed, immune, nervous, gastrointestinal and reproductive tissues. Therefore, SYNT appears to play a role in an immune disorder such as inflammation, actinic keratosis, acquired immunodeficiency syndrome (AIDS),

- 5 Addison's disease, adult respiratory distress syndrome, allergies, ankylosing spondylitis, amyloidosis, anemia, arteriosclerosis, asthma, atherosclerosis, autoimmune hemolytic anemia, autoimmune thyroiditis, bronchitis, bursitis, cholecystitis, cirrhosis, contact dermatitis, Crohn's disease, atopic dermatitis, dermatomyositis, diabetes mellitus, emphysema, erythroblastosis fetalis, erythema nodosum, atrophic gastritis, glomerulonephritis, Goodpasture's syndrome, gout, Graves' disease,
- 10 Hashimoto's thyroiditis, paroxysmal nocturnal hemoglobinuria, hepatitis, hypereosinophilia, irritable bowel syndrome, episodic lymphopenia with lymphocytotoxins, mixed connective tissue disease (MCTD), multiple sclerosis, myasthenia gravis, myocardial or pericardial inflammation, myelofibrosis, osteoarthritis, osteoporosis, pancreatitis, polycythemia vera, polymyositis, psoriasis, Reiter's syndrome, rheumatoid arthritis, scleroderma, Sjögren's syndrome, systemic anaphylaxis,
- 15 systemic lupus erythematosus, systemic sclerosis, primary thrombocythemia, thrombocytopenic purpura, ulcerative colitis, uveitis, Werner syndrome, complications of cancer, hemodialysis, and extracorporeal circulation, trauma, viral, bacterial, fungal, parasitic, protozoal, and helminthic infections, and hematopoietic cancer including lymphoma, leukemia, and myeloma; a neuronal disorder, such as akathisia, Alzheimer's disease, amnesia, amyotrophic lateral sclerosis, bipolar
- 20 disorder, catatonia, cerebral neoplasms, dementia, depression, diabetic neuropathy, Down's syndrome, tardive dyskinesia, dystonias, epilepsy, Huntington's disease, peripheral neuropathy, multiple sclerosis, neurofibromatosis, Parkinson's disease, paranoid psychoses, postherpetic neuralgia, schizophrenia, and Tourette's disorder; a reproductive disorder, such as a disorder of prolactin production, infertility, including tubal disease, ovulatory defects, and endometriosis, a disruption of
- 25 the estrous cycle, a disruption of the menstrual cycle, polycystic ovary syndrome, ovarian hyperstimulation syndrome, an endometrial or ovarian tumor, a uterine fibroid, autoimmune disorders, an ectopic pregnancy, and teratogenesis; cancer of the breast, fibrocystic breast disease, and galactorrhea; a disruption of spermatogenesis, abnormal sperm physiology, cancer of the testis, cancer of the prostate, benign prostatic hyperplasia, prostatitis, Peyronie's disease, impotence,
- 30 carcinoma of the male breast, and gynecomastia; and a cell proliferative disorder, such as actinic keratosis, arteriosclerosis, atherosclerosis, bursitis, cirrhosis, hepatitis, mixed connective tissue disease (MCTD), myelofibrosis, paroxysmal nocturnal hemoglobinuria, polycythemia vera, psoriasis, primary thrombocythemia, and cancers including adenocarcinoma, leukemia, lymphoma, melanoma, myeloma, sarcoma, teratocarcinoma, and, in particular, a cancer of the adrenal gland, bladder, bone,
- 35 bone marrow, brain, breast, cervix, gall bladder, ganglia, gastrointestinal tract, heart, kidney, liver, lung, muscle, ovary, pancreas, parathyroid, penis, prostate, salivary glands, skin, spleen, testis,

thymus, thyroid, and uterus.

In another embodiment, a vector capable of expressing SYNT or a fragment or derivative thereof may be administered to a subject to treat or prevent a disorder associated with decreased expression or activity of SYNT including, but not limited to, those described above.

5 In a further embodiment, a pharmaceutical composition comprising a substantially purified SYNT in conjunction with a suitable pharmaceutical carrier may be administered to a subject to treat or prevent a disorder associated with decreased expression or activity of SYNT including, but not limited to, those provided above.

In still another embodiment, an agonist which modulates the activity of SYNT may be  
10 administered to a subject to treat or prevent a disorder associated with decreased expression or activity of SYNT including, but not limited to, those listed above.

In a further embodiment, an antagonist of SYNT may be administered to a subject to treat or prevent a disorder associated with increased expression or activity of SYNT. Examples of such disorders include, but are not limited to, those immune, neuronal, reproductive, and cell proliferative  
15 disorders described above. In one aspect, an antibody which specifically binds SYNT may be used directly as an antagonist or indirectly as a targeting or delivery mechanism for bringing a pharmaceutical agent to cells or tissues which express SYNT.

In an additional embodiment, a vector expressing the complement of the polynucleotide encoding SYNT may be administered to a subject to treat or prevent a disorder associated with  
20 increased expression or activity of SYNT including, but not limited to, those described above.

In other embodiments, any of the proteins, antagonists, antibodies, agonists, complementary sequences, or vectors of the invention may be administered in combination with other appropriate therapeutic agents. Selection of the appropriate agents for use in combination therapy may be made by one of ordinary skill in the art, according to conventional pharmaceutical principles. The  
25 combination of therapeutic agents may act synergistically to effect the treatment or prevention of the various disorders described above. Using this approach, one may be able to achieve therapeutic efficacy with lower dosages of each agent, thus reducing the potential for adverse side effects.

An antagonist of SYNT may be produced using methods which are generally known in the art. In particular, purified SYNT may be used to produce antibodies or to screen libraries of  
30 pharmaceutical agents to identify those which specifically bind SYNT. Antibodies to SYNT may also be generated using methods that are well known in the art. Such antibodies may include, but are not limited to, polyclonal, monoclonal, chimeric, and single chain antibodies, Fab fragments, and fragments produced by a Fab expression library. Neutralizing antibodies (i.e., those which inhibit dimer formation) are generally preferred for therapeutic use.

35 For the production of antibodies, various hosts including goats, rabbits, rats, mice, humans, and others may be immunized by injection with SYNT or with any fragment or oligopeptide thereof



which has immunogenic properties. Depending on the host species, various adjuvants may be used to increase immunological response. Such adjuvants include, but are not limited to, Freund's, mineral gels such as aluminum hydroxide, and surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, KLH, and dinitrophenol. Among adjuvants used in humans, BCG (bacilli Calmette-Guerin) and Cornebacterium parvum are especially preferable.

It is preferred that the oligopeptides, peptides, or fragments used to induce antibodies to SYNT have an amino acid sequence consisting of at least about 5 amino acids, and generally will consist of at least about 10 amino acids. It is also preferable that these oligopeptides, peptides, or fragments are identical to a portion of the amino acid sequence of the natural protein. Short stretches of SYNT amino acids may be fused with those of another protein, such as KLH, and antibodies to the chimeric molecule may be produced.

Monoclonal antibodies to SYNT may be prepared using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include, but are not limited to, the hybridoma technique, the human B-cell hybridoma technique, and the EBV-hybridoma technique. (See, e.g., Kohler, G. et al. (1975) Nature 256:495-497; Kozbor, D. et al. (1985) J. Immunol. Methods 81:31-42; Cote, R.J. et al. (1983) Proc. Natl. Acad. Sci. USA 80:2026-2030; and Cole, S.P. et al. (1984) Mol. Cell Biol. 62:109-120.)

In addition, techniques developed for the production of "chimeric antibodies," such as the splicing of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity, can be used. (See, e.g., Morrison, S.L. et al. (1984) Proc. Natl. Acad. Sci. USA 81:6851-6855; Neuberger, M.S. et al. (1984) Nature 312:604-608; and Takeda, S. et al. (1985) Nature 314:452-454.) Alternatively, techniques described for the production of single chain antibodies may be adapted, using methods known in the art, to produce SYNT-specific single chain antibodies. Antibodies with related specificity, but of distinct idiotypic composition, may be generated by chain shuffling from random combinatorial immunoglobulin libraries. (See, e.g., Burton, D.R. (1991) Proc. Natl. Acad. Sci. USA 88:10134-10137.)

Antibodies may also be produced by inducing in vivo production in the lymphocyte population or by screening immunoglobulin libraries or panels of highly specific binding reagents as disclosed in the literature. (See, e.g., Orlandi, R. et al. (1989) Proc. Natl. Acad. Sci. USA 86:3833-3837; Winter, G. et al. (1991) Nature 349:293-299.)

Antibody fragments which contain specific binding sites for SYNT may also be generated. For example, such fragments include, but are not limited to, F(ab')<sub>2</sub> fragments produced by pepsin digestion of the antibody molecule and Fab fragments generated by reducing the disulfide bridges of the F(ab')<sub>2</sub> fragments. Alternatively, Fab expression libraries may be constructed to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity. (See, e.g., Huse, W.D. et al. (1989) Science 246:1275-1281.)

Various immunoassays may be used for screening to identify antibodies having the desired specificity. Numerous protocols for competitive binding or immunoradiometric assays using either polyclonal or monoclonal antibodies with established specificities are well known in the art. Such immunoassays typically involve the measurement of complex formation between SYNT and its  
5 specific antibody. A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering SYNT epitopes is generally used, but a competitive binding assay may also be employed (Pound, supra).

Various methods such as Scatchard analysis in conjunction with radioimmunoassay techniques may be used to assess the affinity of antibodies for SYNT. Affinity is expressed as an  
10 association constant,  $K_a$ , which is defined as the molar concentration of SYNT-antibody complex divided by the molar concentrations of free antigen and free antibody under equilibrium conditions. The  $K_a$  determined for a preparation of polyclonal antibodies, which are heterogeneous in their affinities for multiple SYNT epitopes, represents the average affinity, or avidity, of the antibodies for SYNT. The  $K_a$  determined for a preparation of monoclonal antibodies, which are monospecific for a  
15 particular SYNT epitope, represents a true measure of affinity. High-affinity antibody preparations with  $K_a$  ranging from about  $10^9$  to  $10^{12}$  L/mole are preferred for use in immunoassays in which the SYNT-antibody complex must withstand rigorous manipulations. Low-affinity antibody preparations with  $K_a$  ranging from about  $10^6$  to  $10^7$  L/mole are preferred for use in immunopurification and similar procedures which ultimately require dissociation of SYNT, preferably in active form, from the  
20 antibody (Catty, D. (1988) Antibodies, Volume I: A Practical Approach, IRL Press, Washington DC; Liddell, J.E. and A. Cryer (1991) A Practical Guide to Monoclonal Antibodies, John Wiley & Sons, New York NY).

The titer and avidity of polyclonal antibody preparations may be further evaluated to determine the quality and suitability of such preparations for certain downstream applications. For  
25 example, a polyclonal antibody preparation containing at least 1-2 mg specific antibody/ml, preferably 5-10 mg specific antibody/ml, is generally employed in procedures requiring precipitation of SYNT-antibody complexes. Procedures for evaluating antibody specificity, titer, and avidity, and guidelines for antibody quality and usage in various applications, are generally available. (See, e.g., Catty, supra, and Coligan et al., supra.)

In another embodiment of the invention, the polynucleotides encoding SYNT, or any  
30 fragment or complement thereof, may be used for therapeutic purposes. In one aspect, modifications of gene expression can be achieved by designing complementary sequences or antisense molecules (DNA, RNA, PNA, or modified oligonucleotides) to the coding or regulatory regions of the gene encoding SYNT. Such technology is well known in the art, and antisense oligonucleotides or larger  
35 fragments can be designed from various locations along the coding or control regions of sequences encoding SYNT. (See, e.g., Agrawal, S., ed. (1996) Antisense Therapeutics, Humana Press Inc.,

Totawa NJ.)

In therapeutic use, any gene delivery system suitable for introduction of the antisense sequences into appropriate target cells can be used. Antisense sequences can be delivered intracellularly in the form of an expression plasmid which, upon transcription, produces a sequence complementary to at least a portion of the cellular sequence encoding the target protein. (See, e.g., Slater, J.E. et al. (1998) *J. Allergy Clin. Immunol.* 102(3):469-475; and Scanlon, K.J. et al. (1995) 9(13):1288-1296.) Antisense sequences can also be introduced intracellularly through the use of viral vectors, such as retrovirus and adeno-associated virus vectors. (See, e.g., Miller, A.D. (1990) *Blood* 76:271; Ausubel, *supra*; Uckert, W. and W. Walther (1994) *Pharmacol. Ther.* 63(3):323-347.) Other gene delivery mechanisms include liposome-derived systems, artificial viral envelopes, and other systems known in the art. (See, e.g., Rossi, J.J. (1995) *Br. Med. Bull.* 51(1):217-225; Boado, R.J. et al. (1998) *J. Pharm. Sci.* 87(11):1308-1315; and Morris, M.C. et al. (1997) *Nucleic Acids Res.* 25(14):2730-2736.)

In another embodiment of the invention, polynucleotides encoding SYNT may be used for somatic or germline gene therapy. Gene therapy may be performed to (i) correct a genetic deficiency (e.g., in the cases of severe combined immunodeficiency (SCID)-X1 disease characterized by X-linked inheritance (Cavazzana-Calvo, M. et al. (2000) *Science* 288:669-672), severe combined immunodeficiency syndrome associated with an inherited adenosine deaminase (ADA) deficiency (Blaese, R.M. et al. (1995) *Science* 270:475-480; Bordignon, C. et al. (1995) *Science* 270:470-475), cystic fibrosis (Zabner, J. et al. (1993) *Cell* 75:207-216; Crystal, R.G. et al. (1995) *Hum. Gene Therapy* 6:643-666; Crystal, R.G. et al. (1995) *Hum. Gene Therapy* 6:667-703), thalassamias, familial hypercholesterolemia, and hemophilia resulting from Factor VIII or Factor IX deficiencies (Crystal, R.G. (1995) *Science* 270:404-410; Verma, I.M. and Somia, N. (1997) *Nature* 389:239-242)), (ii) express a conditionally lethal gene product (e.g., in the case of cancers which result from unregulated cell proliferation), or (iii) express a protein which affords protection against intracellular parasites (e.g., against human retroviruses, such as human immunodeficiency virus (HIV) (Baltimore, D. (1988) *Nature* 335:395-396; Poeschla, E. et al. (1996) *Proc. Natl. Acad. Sci. USA.* 93:11395-11399), hepatitis B or C virus (HBV, HCV); fungal parasites, such as Candida albicans and Paracoccidioides brasiliensis; and protozoan parasites such as Plasmodium falciparum and Trypanosoma cruzi). In the case where a genetic deficiency in SYNT expression or regulation causes disease, the expression of SYNT from an appropriate population of transduced cells may alleviate the clinical manifestations caused by the genetic deficiency.

In a further embodiment of the invention, diseases or disorders caused by deficiencies in SYNT are treated by constructing mammalian expression vectors encoding SYNT and introducing these vectors by mechanical means into SYNT-deficient cells. Mechanical transfer technologies for use with cells in vivo or ex vitro include (i) direct DNA microinjection into individual cells, (ii)

ballistic gold particle delivery, (iii) liposome-mediated transfection, (iv) receptor-mediated gene transfer, and (v) the use of DNA transposons (Morgan, R.A. and W.F. Anderson (1993) *Annu. Rev. Biochem.* 62:191-217; Ivics, Z. (1997) *Cell* 91:501-510; Boulay, J-L. and H. Récipon (1998) *Curr. Opin. Biotechnol.* 9:445-450).

5           Expression vectors that may be effective for the expression of SYNT include, but are not limited to, the PCDNA 3.1, EPITAG, PRCCMV2, PREP, PVAX vectors (Invitrogen, Carlsbad CA), PCMV-SCRIPT, PCMV-TAG, PEGSH/PERV (Stratagene, La Jolla CA), and PTET-OFF, PTET-ON, PTRE2, PTRE2-LUC, PTK-HYG (Clontech, Palo Alto CA). SYNT may be expressed using (i) a constitutively active promoter, (e.g., from cytomegalovirus (CMV), Rous sarcoma virus  
10 (RSV), SV40 virus, thymidine kinase (TK), or  $\beta$ -actin genes), (ii) an inducible promoter (e.g., the tetracycline-regulated promoter (Gossen, M. and H. Bujard (1992) *Proc. Natl. Acad. Sci. USA* 89:5547-5551; Gossen, M. et al. (1995) *Science* 268:1766-1769; Rossi, F.M.V. and H.M. Blau (1998) *Curr. Opin. Biotechnol.* 9:451-456), commercially available in the T-REX plasmid (Invitrogen)); the ecdysone-inducible promoter (available in the plasmids PVGRXR and PIND; Invitrogen); the  
15 FK506/rapamycin inducible promoter; or the RU486/mifepristone inducible promoter (Rossi, F.M.V. and H.M. Blau, *supra*), or (iii) a tissue-specific promoter or the native promoter of the endogenous gene encoding SYNT from a normal individual.

          Commercially available liposome transformation kits (e.g., the PERFECT LIPID TRANSFECTION KIT, available from Invitrogen) allow one with ordinary skill in the art to deliver  
20 polynucleotides to target cells in culture and require minimal effort to optimize experimental parameters. In the alternative, transformation is performed using the calcium phosphate method (Graham, F.L. and A.J. Eb (1973) *Virology* 52:456-467), or by electroporation (Neumann, E. et al. (1982) *EMBO J.* 1:841-845). The introduction of DNA to primary cells requires modification of these standardized mammalian transfection protocols.

25           In another embodiment of the invention, diseases or disorders caused by genetic defects with respect to SYNT expression are treated by constructing a retrovirus vector consisting of (i) the polynucleotide encoding SYNT under the control of an independent promoter or the retrovirus long terminal repeat (LTR) promoter, (ii) appropriate RNA packaging signals, and (iii) a Rev-responsive element (RRE) along with additional retrovirus *cis*-acting RNA sequences and coding sequences  
30 required for efficient vector propagation. Retrovirus vectors (e.g., PFB and PFBNEO) are commercially available (Stratagene) and are based on published data (Riviere, I. et al. (1995) *Proc. Natl. Acad. Sci. USA* 92:6733-6737), incorporated by reference herein. The vector is propagated in an appropriate vector producing cell line (VPCL) that expresses an envelope gene with a tropism for receptors on the target cells or a promiscuous envelope protein such as VSVg (Armentano, D. et al.  
35 (1987) *J. Virol.* 61:1647-1650; Bender, M.A. et al. (1987) *J. Virol.* 61:1639-1646; Adam, M.A. and A.D. Miller (1988) *J. Virol.* 62:3802-3806; Dull, T. et al. (1998) *J. Virol.* 72:8463-8471; Zufferey, R.

et al. (1998) J. Virol. 72:9873-9880). U.S. Patent Number 5,910,434 to Rigg ("Method for obtaining retrovirus packaging cell lines producing high transducing efficiency retroviral supernatant") discloses a method for obtaining retrovirus packaging cell lines and is hereby incorporated by reference. Propagation of retrovirus vectors, transduction of a population of cells (e.g., CD4<sup>+</sup> T-cells), and the return of transduced cells to a patient are procedures well known to persons skilled in the art of gene therapy and have been well documented (Ranga, U. et al. (1997) J. Virol. 71:7020-7029; Bauer, G. et al. (1997) Blood 89:2259-2267; Bonyhadi, M.L. (1997) J. Virol. 71:4707-4716; Ranga, U. et al. (1998) Proc. Natl. Acad. Sci. USA 95:1201-1206; Su, L. (1997) Blood 89:2283-2290).

In the alternative, an adenovirus-based gene therapy delivery system is used to deliver polynucleotides encoding SYNT to cells which have one or more genetic abnormalities with respect to the expression of SYNT. The construction and packaging of adenovirus-based vectors are well known to those with ordinary skill in the art. Replication defective adenovirus vectors have proven to be versatile for importing genes encoding immunoregulatory proteins into intact islets in the pancreas (Csete, M.E. et al. (1995) Transplantation 27:263-268). Potentially useful adenoviral vectors are described in U.S. Patent Number 5,707,618 to Armentano ("Adenovirus vectors for gene therapy"), hereby incorporated by reference. For adenoviral vectors, see also Antinozzi, P.A. et al. (1999) Annu. Rev. Nutr. 19:511-544; and Verma, I.M. and N. Somia (1997) Nature 18:389:239-242, both incorporated by reference herein.

In another alternative, a herpes-based, gene therapy delivery system is used to deliver polynucleotides encoding SYNT to target cells which have one or more genetic abnormalities with respect to the expression of SYNT. The use of herpes simplex virus (HSV)-based vectors may be especially valuable for introducing SYNT to cells of the central nervous system, for which HSV has a tropism. The construction and packaging of herpes-based vectors are well known to those with ordinary skill in the art. A replication-competent herpes simplex virus (HSV) type 1-based vector has been used to deliver a reporter gene to the eyes of primates (Liu, X. et al. (1999) Exp. Eye Res. 169:385-395). The construction of a HSV-1 virus vector has also been disclosed in detail in U.S. Patent Number 5,804,413 to DeLuca ("Herpes simplex virus strains for gene transfer"), which is hereby incorporated by reference. U.S. Patent Number 5,804,413 teaches the use of recombinant HSV d92 which consists of a genome containing at least one exogenous gene to be transferred to a cell under the control of the appropriate promoter for purposes including human gene therapy. Also taught by this patent are the construction and use of recombinant HSV strains deleted for ICP4, ICP27 and ICP22. For HSV vectors, see also Goins, W.F. et al. (1999) J. Virol. 73:519-532 and Xu, H. et al. (1994) Dev. Biol. 163:152-161, hereby incorporated by reference. The manipulation of cloned herpesvirus sequences, the generation of recombinant virus following the transfection of multiple plasmids containing different segments of the large herpesvirus genomes, the growth and propagation

of herpesvirus, and the infection of cells with herpesvirus are techniques well known to those of ordinary skill in the art.

In another alternative, an alphavirus (positive, single-stranded RNA virus) vector is used to deliver polynucleotides encoding SYNT to target cells. The biology of the prototypic alphavirus, Semliki Forest Virus (SFV), has been studied extensively and gene transfer vectors have been based on the SFV genome (Garoff, H. and K.-J. Li (1998) *Curr. Opin. Biotech.* 9:464-469). During alphavirus RNA replication, a subgenomic RNA is generated that normally encodes the viral capsid proteins. This subgenomic RNA replicates to higher levels than the full-length genomic RNA, resulting in the overproduction of capsid proteins relative to the viral proteins with enzymatic activity (e.g., protease and polymerase). Similarly, inserting the coding sequence for SYNT into the alphavirus genome in place of the capsid-coding region results in the production of a large number of SYNT-coding RNAs and the synthesis of high levels of SYNT in vector transduced cells. While alphavirus infection is typically associated with cell lysis within a few days, the ability to establish a persistent infection in hamster normal kidney cells (BHK-21) with a variant of Sindbis virus (SIN) indicates that the lytic replication of alphaviruses can be altered to suit the needs of the gene therapy application (Dryga, S.A. et al. (1997) *Virology* 228:74-83). The wide host range of alphaviruses will allow the introduction of SYNT into a variety of cell types. The specific transduction of a subset of cells in a population may require the sorting of cells prior to transduction. The methods of manipulating infectious cDNA clones of alphaviruses, performing alphavirus cDNA and RNA transfections, and performing alphavirus infections, are well known to those with ordinary skill in the art.

Oligonucleotides derived from the transcription initiation site, e.g., between about positions -10 and +10 from the start site, may also be employed to inhibit gene expression. Similarly, inhibition can be achieved using triple helix base-pairing methodology. Triple helix pairing is useful because it causes inhibition of the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors, or regulatory molecules. Recent therapeutic advances using triplex DNA have been described in the literature. (See, e.g., Gee, J.E. et al. (1994) in Huber, B.E. and B.I. Carr, Molecular and Immunologic Approaches, Futura Publishing, Mt. Kisco NY, pp. 163-177.) A complementary sequence or antisense molecule may also be designed to block translation of mRNA by preventing the transcript from binding to ribosomes.

Ribozymes, enzymatic RNA molecules, may also be used to catalyze the specific cleavage of RNA. The mechanism of ribozyme action involves sequence-specific hybridization of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. For example, engineered hammerhead motif ribozyme molecules may specifically and efficiently catalyze endonucleolytic cleavage of sequences encoding SYNT.

Specific ribozyme cleavage sites within any potential RNA target are initially identified by

scanning the target molecule for ribozyme cleavage sites, including the following sequences: GUA, GUU, and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides, corresponding to the region of the target gene containing the cleavage site, may be evaluated for secondary structural features which may render the oligonucleotide inoperable. The suitability of candidate targets may also be evaluated by testing accessibility to hybridization with complementary oligonucleotides using ribonuclease protection assays.

Complementary ribonucleic acid molecules and ribozymes of the invention may be prepared by any method known in the art for the synthesis of nucleic acid molecules. These include techniques for chemically synthesizing oligonucleotides such as solid phase phosphoramidite chemical synthesis.

Alternatively, RNA molecules may be generated by *in vitro* and *in vivo* transcription of DNA sequences encoding SYNT. Such DNA sequences may be incorporated into a wide variety of vectors with suitable RNA polymerase promoters such as T7 or SP6. Alternatively, these cDNA constructs that synthesize complementary RNA, constitutively or inducibly, can be introduced into cell lines, cells, or tissues.

RNA molecules may be modified to increase intracellular stability and half-life. Possible modifications include, but are not limited to, the addition of flanking sequences at the 5' and/or 3' ends of the molecule, or the use of phosphorothioate or 2'-O-methyl rather than phosphodiesterase linkages within the backbone of the molecule. This concept is inherent in the production of PNAs and can be extended in all of these molecules by the inclusion of nontraditional bases such as inosine, queosine, and wybutosine, as well as acetyl-, methyl-, thio-, and similarly modified forms of adenine, cytidine, guanine, thymine, and uridine which are not as easily recognized by endogenous endonucleases.

An additional embodiment of the invention encompasses a method for screening for a compound which is effective in altering expression of a polynucleotide encoding SYNT. Compounds which may be effective in altering expression of a specific polynucleotide may include, but are not limited to, oligonucleotides, antisense oligonucleotides, triple helix-forming oligonucleotides, transcription factors and other polypeptide transcriptional regulators, and non-macromolecular chemical entities which are capable of interacting with specific polynucleotide sequences. Effective compounds may alter polynucleotide expression by acting as either inhibitors or promoters of polynucleotide expression. Thus, in the treatment of disorders associated with increased SYNT expression or activity, a compound which specifically inhibits expression of the polynucleotide encoding SYNT may be therapeutically useful, and in the treatment of disorders associated with decreased SYNT expression or activity, a compound which specifically promotes expression of the polynucleotide encoding SYNT may be therapeutically useful.

At least one, and up to a plurality, of test compounds may be screened for effectiveness in altering expression of a specific polynucleotide. A test compound may be obtained by any method

commonly known in the art, including chemical modification of a compound known to be effective in altering polynucleotide expression; selection from an existing, commercially-available or proprietary library of naturally-occurring or non-natural chemical compounds; rational design of a compound based on chemical and/or structural properties of the target polynucleotide; and selection from a library of chemical compounds created combinatorially or randomly. A sample comprising a polynucleotide encoding SYNT is exposed to at least one test compound thus obtained. The sample may comprise, for example, an intact or permeabilized cell, or an in vitro cell-free or reconstituted biochemical system. Alterations in the expression of a polynucleotide encoding SYNT are assayed by any method commonly known in the art. Typically, the expression of a specific nucleotide is detected by hybridization with a probe having a nucleotide sequence complementary to the sequence of the polynucleotide encoding SYNT. The amount of hybridization may be quantified, thus forming the basis for a comparison of the expression of the polynucleotide both with and without exposure to one or more test compounds. Detection of a change in the expression of a polynucleotide exposed to a test compound indicates that the test compound is effective in altering the expression of the polynucleotide. A screen for a compound effective in altering expression of a specific polynucleotide can be carried out, for example, using a Schizosaccharomyces pombe gene expression system (Atkins, D. et al. (1999) U.S. Patent No. 5,932,435; Arndt, G.M. et al. (2000) Nucleic Acids Res. 28:E15) or a human cell line such as HeLa cell (Clarke, M.L. et al. (2000) Biochem. Biophys. Res. Commun. 268:8-13). A particular embodiment of the present invention involves screening a combinatorial library of oligonucleotides (such as deoxyribonucleotides, ribonucleotides, peptide nucleic acids, and modified oligonucleotides) for antisense activity against a specific polynucleotide sequence (Bruce, T.W. et al. (1997) U.S. Patent No. 5,686,242; Bruce, T.W. et al. (2000) U.S. Patent No. 6,022,691).

Many methods for introducing vectors into cells or tissues are available and equally suitable for use in vivo, in vitro, and ex vivo. For ex vivo therapy, vectors may be introduced into stem cells taken from the patient and clonally propagated for autologous transplant back into that same patient. Delivery by transfection, by liposome injections, or by polycationic amino polymers may be achieved using methods which are well known in the art. (See, e.g., Goldman, C.K. et al. (1997) Nat. Biotechnol. 15:462-466.)

Any of the therapeutic methods described above may be applied to any subject in need of such therapy, including, for example, mammals such as humans, dogs, cats, cows, horses, rabbits, and monkeys.

An additional embodiment of the invention relates to the administration of a pharmaceutical composition which generally comprises an active ingredient formulated with a pharmaceutically acceptable excipient. Excipients may include, for example, sugars, starches, celluloses, gums, and proteins. Various formulations are commonly known and are thoroughly discussed in the latest



edition of Remington's Pharmaceutical Sciences (Maack Publishing, Easton PA). Such pharmaceutical compositions may consist of SYNT, antibodies to SYNT, and mimetics, agonists, antagonists, or inhibitors of SYNT.

5 The pharmaceutical compositions utilized in this invention may be administered by any number of routes including, but not limited to, oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, pulmonary, transdermal, subcutaneous, intraperitoneal, intranasal, enteral, topical, sublingual, or rectal means.

Pharmaceutical compositions for pulmonary administration may be prepared in liquid or dry powder form. These compositions are generally aerosolized immediately prior to inhalation by the patient. In the case of small molecules (e.g. traditional low molecular weight organic drugs), aerosol delivery of fast-acting formulations is well-known in the art. In the case of macromolecules (e.g. larger peptides and proteins), recent developments in the field of pulmonary delivery via the alveolar region of the lung have enabled the practical delivery of drugs such as insulin to blood circulation (see, e.g., Patton, J.S. et al., U.S. Patent No. 5,997,848). Pulmonary delivery has the advantage of administration without needle injection, and obviates the need for potentially toxic penetration enhancers.

Pharmaceutical compositions suitable for use in the invention include compositions wherein the active ingredients are contained in an effective amount to achieve the intended purpose. The determination of an effective dose is well within the capability of those skilled in the art.

20 Specialized forms of pharmaceutical compositions may be prepared for direct intracellular delivery of macromolecules comprising SYNT or fragments thereof. For example, liposome preparations containing a cell-impermeable macromolecule may promote cell fusion and intracellular delivery of the macromolecule. Alternatively, SYNT or a fragment thereof may be joined to a short cationic N-terminal portion from the HIV Tat-1 protein. Fusion proteins thus generated have been found to transduce into the cells of all tissues, including the brain, in a mouse model system (Schwarze, S.R. et al. (1999) Science 285:1569-1572).

For any compound, the therapeutically effective dose can be estimated initially either in cell culture assays, e.g., of neoplastic cells, or in animal models such as mice, rats, rabbits, dogs, monkeys, or pigs. An animal model may also be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans.

A therapeutically effective dose refers to that amount of active ingredient, for example SYNT or fragments thereof, antibodies of SYNT, and agonists, antagonists or inhibitors of SYNT, which ameliorates the symptoms or condition. Therapeutic efficacy and toxicity may be determined by standard pharmaceutical procedures in cell cultures or with experimental animals, such as by calculating the ED<sub>50</sub> (the dose therapeutically effective in 50% of the population) or LD<sub>50</sub> (the dose

lethal to 50% of the population) statistics. The dose ratio of toxic to therapeutic effects is the therapeutic index, which can be expressed as the  $LD_{50}/ED_{50}$  ratio. Pharmaceutical compositions which exhibit large therapeutic indices are preferred. The data obtained from cell culture assays and animal studies are used to formulate a range of dosage for human use. The dosage contained in such compositions is preferably within a range of circulating concentrations that includes the  $ED_{50}$  with little or no toxicity. The dosage varies within this range depending upon the dosage form employed, the sensitivity of the patient, and the route of administration.

The exact dosage will be determined by the practitioner, in light of factors related to the subject requiring treatment. Dosage and administration are adjusted to provide sufficient levels of the active moiety or to maintain the desired effect. Factors which may be taken into account include the severity of the disease state, the general health of the subject, the age, weight, and gender of the subject, time and frequency of administration, drug combination(s), reaction sensitivities, and response to therapy. Long-acting pharmaceutical compositions may be administered every 3 to 4 days, every week, or biweekly depending on the half-life and clearance rate of the particular formulation.

Normal dosage amounts may vary from about 0.1  $\mu$ g to 100,000  $\mu$ g, up to a total dose of about 1 gram, depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature and generally available to practitioners in the art. Those skilled in the art will employ different formulations for nucleotides than for proteins or their inhibitors. Similarly, delivery of polynucleotides or polypeptides will be specific to particular cells, conditions, locations, etc.

## DIAGNOSTICS

In another embodiment, antibodies which specifically bind SYNT may be used for the diagnosis of disorders characterized by expression of SYNT, or in assays to monitor patients being treated with SYNT or agonists, antagonists, or inhibitors of SYNT. Antibodies useful for diagnostic purposes may be prepared in the same manner as described above for therapeutics. Diagnostic assays for SYNT include methods which utilize the antibody and a label to detect SYNT in human body fluids or in extracts of cells or tissues. The antibodies may be used with or without modification, and may be labeled by covalent or non-covalent attachment of a reporter molecule. A wide variety of reporter molecules, several of which are described above, are known in the art and may be used.

A variety of protocols for measuring SYNT, including ELISAs, RIAs, and FACS, are known in the art and provide a basis for diagnosing altered or abnormal levels of SYNT expression. Normal or standard values for SYNT expression are established by combining body fluids or cell extracts taken from normal mammalian subjects, for example, human subjects, with antibody to SYNT under conditions suitable for complex formation. The amount of standard complex formation may be quantitated by various methods, such as photometric means. Quantities of SYNT expressed in

subject, control, and disease samples from biopsied tissues are compared with the standard values. Deviation between standard and subject values establishes the parameters for diagnosing disease.

In another embodiment of the invention, the polynucleotides encoding SYNT may be used for diagnostic purposes. The polynucleotides which may be used include oligonucleotide sequences, complementary RNA and DNA molecules, and PNAs. The polynucleotides may be used to detect and quantify gene expression in biopsied tissues in which expression of SYNT may be correlated with disease. The diagnostic assay may be used to determine absence, presence, and excess expression of SYNT, and to monitor regulation of SYNT levels during therapeutic intervention.

In one aspect, hybridization with PCR probes which are capable of detecting polynucleotide sequences, including genomic sequences, encoding SYNT or closely related molecules may be used to identify nucleic acid sequences which encode SYNT. The specificity of the probe, whether it is made from a highly specific region, e.g., the 5' regulatory region, or from a less specific region, e.g., a conserved motif, and the stringency of the hybridization or amplification will determine whether the probe identifies only naturally occurring sequences encoding SYNT, allelic variants, or related sequences.

Probes may also be used for the detection of related sequences, and may have at least 50% sequence identity to any of the SYNT encoding sequences. The hybridization probes of the subject invention may be DNA or RNA and may be derived from the sequence of SEQ ID NO:16-30 or from genomic sequences including promoters, enhancers, and introns of the SYNT gene.

Means for producing specific hybridization probes for DNAs encoding SYNT include the cloning of polynucleotide sequences encoding SYNT or SYNT derivatives into vectors for the production of mRNA probes. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes *in vitro* by means of the addition of the appropriate RNA polymerases and the appropriate labeled nucleotides. Hybridization probes may be labeled by a variety of reporter groups, for example, by radionuclides such as  $^{32}\text{P}$  or  $^{35}\text{S}$ , or by enzymatic labels, such as alkaline phosphatase coupled to the probe via avidin/biotin coupling systems, and the like.

Polynucleotide sequences encoding SYNT may be used for the diagnosis of disorders associated with expression of SYNT. Examples of such disorders include, but are not limited to, an immune disorder such as inflammation, actinic keratosis, acquired immunodeficiency syndrome (AIDS), Addison's disease, adult respiratory distress syndrome, allergies, ankylosing spondylitis, amyloidosis, anemia, arteriosclerosis, asthma, atherosclerosis, autoimmune hemolytic anemia, autoimmune thyroiditis, bronchitis, bursitis, cholecystitis, cirrhosis, contact dermatitis, Crohn's disease, atopic dermatitis, dermatomyositis, diabetes mellitus, emphysema, erythroblastosis fetalis, erythema nodosum, atrophic gastritis, glomerulonephritis, Goodpasture's syndrome, gout, Graves' disease, Hashimoto's thyroiditis, paroxysmal nocturnal hemoglobinuria, hepatitis, hypereosinophilia, irritable bowel syndrome, episodic lymphopenia with lymphocytotoxins, mixed connective tissue

disease (MCTD), multiple sclerosis, myasthenia gravis, myocardial or pericardial inflammation, myelofibrosis, osteoarthritis, osteoporosis, pancreatitis, polycythemia vera, polymyositis, psoriasis, Reiter's syndrome, rheumatoid arthritis, scleroderma, Sjögren's syndrome, systemic anaphylaxis, systemic lupus erythematosus, systemic sclerosis, primary thrombocythemia, thrombocytopenic purpura, ulcerative colitis, uveitis, Werner syndrome, complications of cancer, hemodialysis, and extracorporeal circulation, trauma, viral, bacterial, fungal, parasitic, protozoal, and helminthic infections, and hematopoietic cancer including lymphoma, leukemia, and myeloma; a neuronal disorder, such as akathisia, Alzheimer's disease, amnesia, amyotrophic lateral sclerosis, bipolar disorder, catatonia, cerebral neoplasms, dementia, depression, diabetic neuropathy, Down's syndrome, tardive dyskinesia, dystonias, epilepsy, Huntington's disease, peripheral neuropathy, multiple sclerosis, neurofibromatosis, Parkinson's disease, paranoid psychoses, postherpetic neuralgia, schizophrenia, and Tourette's disorder; a reproductive disorder, such as a disorder of prolactin production, infertility, including tubal disease, ovulatory defects, and endometriosis, a disruption of the estrous cycle, a disruption of the menstrual cycle, polycystic ovary syndrome, ovarian hyperstimulation syndrome, an endometrial or ovarian tumor, a uterine fibroid, autoimmune disorders, an ectopic pregnancy, and teratogenesis; cancer of the breast, fibrocystic breast disease, and galactorrhea; a disruption of spermatogenesis, abnormal sperm physiology, cancer of the testis, cancer of the prostate, benign prostatic hyperplasia, prostatitis, Peyronie's disease, impotence, carcinoma of the male breast, and gynecomastia; and a cell proliferative disorder, such as actinic keratosis, arteriosclerosis, atherosclerosis, bursitis, cirrhosis, hepatitis, mixed connective tissue disease (MCTD), myelofibrosis, paroxysmal nocturnal hemoglobinuria, polycythemia vera, psoriasis, primary thrombocythemia, and cancers including adenocarcinoma, leukemia, lymphoma, melanoma, myeloma, sarcoma, teratocarcinoma, and, in particular, a cancer of the adrenal gland, bladder, bone, bone marrow, brain, breast, cervix, gall bladder, ganglia, gastrointestinal tract, heart, kidney, liver, lung, muscle, ovary, pancreas, parathyroid, penis, prostate, salivary glands, skin, spleen, testis, thymus, thyroid, and uterus. The polynucleotide sequences encoding SYNT may be used in Southern or northern analysis, dot blot, or other membrane-based technologies; in PCR technologies; in dipstick, pin, and multiformat ELISA-like assays; and in microarrays utilizing fluids or tissues from patients to detect altered SYNT expression. Such qualitative or quantitative methods are well known in the art.

In a particular aspect, the nucleotide sequences encoding SYNT may be useful in assays that detect the presence of associated disorders, particularly those mentioned above. The nucleotide sequences encoding SYNT may be labeled by standard methods and added to a fluid or tissue sample from a patient under conditions suitable for the formation of hybridization complexes. After a suitable incubation period, the sample is washed and the signal is quantified and compared with a standard value. If the amount of signal in the patient sample is significantly altered in comparison to

a control sample then the presence of altered levels of nucleotide sequences encoding SYNT in the sample indicates the presence of the associated disorder. Such assays may also be used to evaluate the efficacy of a particular therapeutic treatment regimen in animal studies, in clinical trials, or to monitor the treatment of an individual patient.

5           In order to provide a basis for the diagnosis of a disorder associated with expression of SYNT, a normal or standard profile for expression is established. This may be accomplished by combining body fluids or cell extracts taken from normal subjects, either animal or human, with a sequence, or a fragment thereof, encoding SYNT, under conditions suitable for hybridization or amplification. Standard hybridization may be quantified by comparing the values obtained from  
10   normal subjects with values from an experiment in which a known amount of a substantially purified polynucleotide is used. Standard values obtained in this manner may be compared with values obtained from samples from patients who are symptomatic for a disorder. Deviation from standard values is used to establish the presence of a disorder.

          Once the presence of a disorder is established and a treatment protocol is initiated,  
15   hybridization assays may be repeated on a regular basis to determine if the level of expression in the patient begins to approximate that which is observed in the normal subject. The results obtained from successive assays may be used to show the efficacy of treatment over a period ranging from several days to months.

          With respect to cancer, the presence of an abnormal amount of transcript (either under- or  
20   overexpressed) in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

25           Additional diagnostic uses for oligonucleotides designed from the sequences encoding SYNT may involve the use of PCR. These oligomers may be chemically synthesized, generated enzymatically, or produced in vitro. Oligomers will preferably contain a fragment of a polynucleotide encoding SYNT, or a fragment of a polynucleotide complementary to the polynucleotide encoding SYNT, and will be employed under optimized conditions for identification of a specific gene or  
30   condition. Oligomers may also be employed under less stringent conditions for detection or quantification of closely related DNA or RNA sequences.

          In a particular aspect, oligonucleotide primers derived from the polynucleotide sequences encoding SYNT may be used to detect single nucleotide polymorphisms (SNPs). SNPs are substitutions, insertions and deletions that are a frequent cause of inherited or acquired genetic  
35   disease in humans. Methods of SNP detection include, but are not limited to, single-stranded conformation polymorphism (SSCP) and fluorescent SSCP (fSSCP) methods. In SSCP,

oligonucleotide primers derived from the polynucleotide sequences encoding SYNT are used to amplify DNA using the polymerase chain reaction (PCR). The DNA may be derived, for example, from diseased or normal tissue, biopsy samples, bodily fluids, and the like. SNPs in the DNA cause differences in the secondary and tertiary structures of PCR products in single-stranded form, and these differences are detectable using gel electrophoresis in non-denaturing gels. In fSCCP, the oligonucleotide primers are fluorescently labeled, which allows detection of the amplimers in high-throughput equipment such as DNA sequencing machines. Additionally, sequence database analysis methods, termed in silico SNP (isSNP), are capable of identifying polymorphisms by comparing the sequence of individual overlapping DNA fragments which assemble into a common consensus sequence. These computer-based methods filter out sequence variations due to laboratory preparation of DNA and sequencing errors using statistical models and automated analyses of DNA sequence chromatograms. In the alternative, SNPs may be detected and characterized by mass spectrometry using, for example, the high throughput MASSARRAY system (Sequenom, Inc., San Diego CA).

Methods which may also be used to quantify the expression of SYNT include radiolabeling or biotinylating nucleotides, coamplification of a control nucleic acid, and interpolating results from standard curves. (See, e.g., Melby, P.C. et al. (1993) *J. Immunol. Methods* 159:235-244; Duplaa, C. et al. (1993) *Anal. Biochem.* 212:229-236.) The speed of quantitation of multiple samples may be accelerated by running the assay in a high-throughput format where the oligomer or polynucleotide of interest is presented in various dilutions and a spectrophotometric or colorimetric response gives rapid quantitation.

In further embodiments, oligonucleotides or longer fragments derived from any of the polynucleotide sequences described herein may be used as elements on a microarray. The microarray can be used in transcript imaging techniques which monitor the relative expression levels of large numbers of genes simultaneously as described in Seilhamer, J.J. et al., "Comparative Gene Transcript Analysis," U.S. Patent No. 5,840,484, incorporated herein by reference. The microarray may also be used to identify genetic variants, mutations, and polymorphisms. This information may be used to determine gene function, to understand the genetic basis of a disorder, to diagnose a disorder, to monitor progression/regression of disease as a function of gene expression, and to develop and monitor the activities of therapeutic agents in the treatment of disease. In particular, this information may be used to develop a pharmacogenomic profile of a patient in order to select the most appropriate and effective treatment regimen for that patient. For example, therapeutic agents which are highly effective and display the fewest side effects may be selected for a patient based on his/her pharmacogenomic profile.

In another embodiment, antibodies specific for SYNT, or SYNT or fragments thereof may be used as elements on a microarray. The microarray may be used to monitor or measure protein-protein interactions, drug-target interactions, and gene expression profiles, as described above.

A particular embodiment relates to the use of the polynucleotides of the present invention to generate a transcript image of a tissue or cell type. A transcript image represents the global pattern of gene expression by a particular tissue or cell type. Global gene expression patterns are analyzed by quantifying the number of expressed genes and their relative abundance under given conditions and at a given time. (See Seilhamer et al., "Comparative Gene Transcript Analysis," U.S. Patent Number 5,840,484, expressly incorporated by reference herein.) Thus a transcript image may be generated by hybridizing the polynucleotides of the present invention or their complements to the totality of transcripts or reverse transcripts of a particular tissue or cell type. In one embodiment, the hybridization takes place in high-throughput format, wherein the polynucleotides of the present invention or their complements comprise a subset of a plurality of elements on a microarray. The resultant transcript image would provide a profile of gene activity.

Transcript images may be generated using transcripts isolated from tissues, cell lines, biopsies, or other biological samples. The transcript image may thus reflect gene expression in vivo, as in the case of a tissue or biopsy sample, or in vitro, as in the case of a cell line.

Transcript images which profile the expression of the polynucleotides of the present invention may also be used in conjunction with in vitro model systems and preclinical evaluation of pharmaceuticals, as well as toxicological testing of industrial and naturally-occurring environmental compounds. All compounds induce characteristic gene expression patterns, frequently termed molecular fingerprints or toxicant signatures, which are indicative of mechanisms of action and toxicity (Nuwaysir, E.F. et al. (1999) Mol. Carcinog. 24:153-159; Steiner, S. and N.L. Anderson (2000) Toxicol. Lett. 112-113:467-471, expressly incorporated by reference herein). If a test compound has a signature similar to that of a compound with known toxicity, it is likely to share those toxic properties. These fingerprints or signatures are most useful and refined when they contain expression information from a large number of genes and gene families. Ideally, a genome-wide measurement of expression provides the highest quality signature. Even genes whose expression is not altered by any tested compounds are important as well, as the levels of expression of these genes are used to normalize the rest of the expression data. The normalization procedure is useful for comparison of expression data after treatment with different compounds. While the assignment of gene function to elements of a toxicant signature aids in interpretation of toxicity mechanisms, knowledge of gene function is not necessary for the statistical matching of signatures which leads to prediction of toxicity. (See, for example, Press Release 00-02 from the National Institute of Environmental Health Sciences, released February 29, 2000, available at <http://www.niehs.nih.gov/oc/news/toxchip.htm>.) Therefore, it is important and desirable in toxicological screening using toxicant signatures to include all expressed gene sequences.

In one embodiment, the toxicity of a test compound is assessed by treating a biological sample containing nucleic acids with the test compound. Nucleic acids that are expressed in the

treated biological sample are hybridized with one or more probes specific to the polynucleotides of the present invention, so that transcript levels corresponding to the polynucleotides of the present invention may be quantified. The transcript levels in the treated biological sample are compared with levels in an untreated biological sample. Differences in the transcript levels between the two samples are indicative of a toxic response caused by the test compound in the treated sample.

Another particular embodiment relates to the use of the polypeptide sequences of the present invention to analyze the proteome of a tissue or cell type. The term proteome refers to the global pattern of protein expression in a particular tissue or cell type. Each protein component of a proteome can be subjected individually to further analysis. Proteome expression patterns, or profiles, are analyzed by quantifying the number of expressed proteins and their relative abundance under given conditions and at a given time. A profile of a cell's proteome may thus be generated by separating and analyzing the polypeptides of a particular tissue or cell type. In one embodiment, the separation is achieved using two-dimensional gel electrophoresis, in which proteins from a sample are separated by isoelectric focusing in the first dimension, and then according to molecular weight by sodium dodecyl sulfate slab gel electrophoresis in the second dimension (Steiner and Anderson, supra). The proteins are visualized in the gel as discrete and uniquely positioned spots, typically by staining the gel with an agent such as Coomassie Blue or silver or fluorescent stains. The optical density of each protein spot is generally proportional to the level of the protein in the sample. The optical densities of equivalently positioned protein spots from different samples, for example, from biological samples either treated or untreated with a test compound or therapeutic agent, are compared to identify any changes in protein spot density related to the treatment. The proteins in the spots are partially sequenced using, for example, standard methods employing chemical or enzymatic cleavage followed by mass spectrometry. The identity of the protein in a spot may be determined by comparing its partial sequence, preferably of at least 5 contiguous amino acid residues, to the polypeptide sequences of the present invention. In some cases, further sequence data may be obtained for definitive protein identification.

A proteomic profile may also be generated using antibodies specific for SYNT to quantify the levels of SYNT expression. In one embodiment, the antibodies are used as elements on a microarray, and protein expression levels are quantified by exposing the microarray to the sample and detecting the levels of protein bound to each array element (Lueking, A. et al. (1999) Anal. Biochem. 270:103-111; Mendoz, L.G. et al. (1999) Biotechniques 27:778-788). Detection may be performed by a variety of methods known in the art, for example, by reacting the proteins in the sample with a thiol- or amino-reactive fluorescent compound and detecting the amount of fluorescence bound at each array element.

Toxicant signatures at the proteome level are also useful for toxicological screening, and should be analyzed in parallel with toxicant signatures at the transcript level. There is a poor



correlation between transcript and protein abundances for some proteins in some tissues (Anderson, N.L. and J. Seilhamer (1997) *Electrophoresis* 18:533-537), so proteome toxicant signatures may be useful in the analysis of compounds which do not significantly affect the transcript image, but which alter the proteomic profile. In addition, the analysis of transcripts in body fluids is difficult, due to rapid degradation of mRNA, so proteomic profiling may be more reliable and informative in such cases.

In another embodiment, the toxicity of a test compound is assessed by treating a biological sample containing proteins with the test compound. Proteins that are expressed in the treated biological sample are separated so that the amount of each protein can be quantified. The amount of each protein is compared to the amount of the corresponding protein in an untreated biological sample. A difference in the amount of protein between the two samples is indicative of a toxic response to the test compound in the treated sample. Individual proteins are identified by sequencing the amino acid residues of the individual proteins and comparing these partial sequences to the polypeptides of the present invention.

In another embodiment, the toxicity of a test compound is assessed by treating a biological sample containing proteins with the test compound. Proteins from the biological sample are incubated with antibodies specific to the polypeptides of the present invention. The amount of protein recognized by the antibodies is quantified. The amount of protein in the treated biological sample is compared with the amount in an untreated biological sample. A difference in the amount of protein between the two samples is indicative of a toxic response to the test compound in the treated sample.

Microarrays may be prepared, used, and analyzed using methods known in the art. (See, e.g., Brennan, T.M. et al. (1995) U.S. Patent No. 5,474,796; Schena, M. et al. (1996) *Proc. Natl. Acad. Sci. USA* 93:10614-10619; Baldeschweiler et al. (1995) PCT application WO95/251116; Shalon, D. et al. (1995) PCT application WO95/35505; Heller, R.A. et al. (1997) *Proc. Natl. Acad. Sci. USA* 94:2150-2155; and Heller, M.J. et al. (1997) U.S. Patent No. 5,605,662.) Various types of microarrays are well known and thoroughly described in DNA Microarrays: A Practical Approach, M. Schena, ed. (1999) Oxford University Press, London, hereby expressly incorporated by reference.

In another embodiment of the invention, nucleic acid sequences encoding SYNT may be used to generate hybridization probes useful in mapping the naturally occurring genomic sequence. Either coding or noncoding sequences may be used, and in some instances, noncoding sequences may be preferable over coding sequences. For example, conservation of a coding sequence among members of a multi-gene family may potentially cause undesired cross hybridization during chromosomal mapping. The sequences may be mapped to a particular chromosome, to a specific region of a chromosome, or to artificial chromosome constructions, e.g., human artificial chromosomes (HACs), yeast artificial chromosomes (YACs), bacterial artificial chromosomes (BACs), bacterial P1

constructions, or single chromosome cDNA libraries. (See, e.g., Harrington, J.J. et al. (1997) *Nat. Genet.* 15:345-355; Price, C.M. (1993) *Blood Rev.* 7:127-134; and Trask, B.J. (1991) *Trends Genet.* 7:149-154.) Once mapped, the nucleic acid sequences of the invention may be used to develop genetic linkage maps, for example, which correlate the inheritance of a disease state with the inheritance of a particular chromosome region or restriction fragment length polymorphism (RFLP). (See, e.g., Lander, E.S. and D. Botstein (1986) *Proc. Natl. Acad. Sci. USA* 83:7353-7357.)

Fluorescent in situ hybridization (FISH) may be correlated with other physical and genetic map data. (See, e.g., Heinz-Ulrich, et al. (1995) in Meyers, supra, pp. 965-968.) Examples of genetic map data can be found in various scientific journals or at the Online Mendelian Inheritance in Man (OMIM) World Wide Web site. Correlation between the location of the gene encoding SYNT on a physical map and a specific disorder, or a predisposition to a specific disorder, may help define the region of DNA associated with that disorder and thus may further positional cloning efforts.

In situ hybridization of chromosomal preparations and physical mapping techniques, such as linkage analysis using established chromosomal markers, may be used for extending genetic maps. Often the placement of a gene on the chromosome of another mammalian species, such as mouse, may reveal associated markers even if the exact chromosomal locus is not known. This information is valuable to investigators searching for disease genes using positional cloning or other gene discovery techniques. Once the gene or genes responsible for a disease or syndrome have been crudely localized by genetic linkage to a particular genomic region, e.g., ataxia-telangiectasia to 11q22-23, any sequences mapping to that area may represent associated or regulatory genes for further investigation. (See, e.g., Gatti, R.A. et al. (1988) *Nature* 336:577-580.) The nucleotide sequence of the instant invention may also be used to detect differences in the chromosomal location due to translocation, inversion, etc., among normal, carrier, or affected individuals.

In another embodiment of the invention, SYNT, its catalytic or immunogenic fragments, or oligopeptides thereof can be used for screening libraries of compounds in any of a variety of drug screening techniques. The fragment employed in such screening may be free in solution, affixed to a solid support, borne on a cell surface, or located intracellularly. The formation of binding complexes between SYNT and the agent being tested may be measured.

Another technique for drug screening provides for high throughput screening of compounds having suitable binding affinity to the protein of interest. (See, e.g., Geysen, et al. (1984) PCT application WO84/03564.) In this method, large numbers of different small test compounds are synthesized on a solid substrate. The test compounds are reacted with SYNT, or fragments thereof, and washed. Bound SYNT is then detected by methods well known in the art. Purified SYNT can also be coated directly onto plates for use in the aforementioned drug screening techniques.

Alternatively, non-neutralizing antibodies can be used to capture the peptide and immobilize it on a solid support.

In another embodiment, one may use competitive drug screening assays in which neutralizing antibodies capable of binding SYNT specifically compete with a test compound for binding SYNT. In this manner, antibodies can be used to detect the presence of any peptide which shares one or more antigenic determinants with SYNT.

5 In additional embodiments, the nucleotide sequences which encode SYNT may be used in any molecular biology techniques that have yet to be developed, provided the new techniques rely on properties of nucleotide sequences that are currently known, including, but not limited to, such properties as the triplet genetic code and specific base pair interactions.

Without further elaboration, it is believed that one skilled in the art can, using the preceding  
10 description, utilize the present invention to its fullest extent. The following preferred specific embodiments are, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever.

The disclosures of all patents, applications, and publications mentioned above and below, in particular U.S. Ser. No. 60/144,992 and U.S. Ser. No. 60/168,858 are hereby expressly incorporated  
15 by reference.

## EXAMPLES

### I. Construction of cDNA Libraries

RNA was purchased from Clontech or isolated from tissues described in Table 4. Some  
20 tissues were homogenized and lysed in guanidinium isothiocyanate, while others were homogenized and lysed in phenol or in a suitable mixture of denaturants, such as TRIZOL (Life Technologies), a monophasic solution of phenol and guanidine isothiocyanate. The resulting lysates were centrifuged over CsCl cushions or extracted with chloroform. RNA was precipitated from the lysates with either isopropanol or sodium acetate and ethanol, or by other routine methods.

25 Phenol extraction and precipitation of RNA were repeated as necessary to increase RNA purity. In some cases, RNA was treated with DNase. For most libraries, poly(A+) RNA was isolated using oligo d(T)-coupled paramagnetic particles (Promega), OLIGOTEX latex particles (QIAGEN, Chatsworth CA), or an OLIGOTEX mRNA purification kit (QIAGEN). Alternatively, RNA was isolated directly from tissue lysates using other RNA isolation kits, e.g., the POLY(A)PURE mRNA  
30 purification kit (Ambion, Austin TX).

In some cases, Stratagene was provided with RNA and constructed the corresponding cDNA libraries. Otherwise, cDNA was synthesized and cDNA libraries were constructed with the UNIZAP vector system (Stratagene) or SUPERScript plasmid system (Life Technologies), using the recommended procedures or similar methods known in the art. (See, e.g., Ausubel, 1997, supra, units  
35 5.1-6.6.) Reverse transcription was initiated using oligo d(T) or random primers. Synthetic oligonucleotide adapters were ligated to double stranded cDNA, and the cDNA was digested with the

appropriate restriction enzyme or enzymes. For most libraries, the cDNA was size-selected (300-1000 bp) using SEPHACRYL S1000, SEPHAROSE CL2B, or SEPHAROSE CL4B column chromatography (Amersham Pharmacia Biotech) or preparative agarose gel electrophoresis. cDNAs were ligated into compatible restriction enzyme sites of the polylinker of a suitable plasmid, e.g.,

5 PBLUESCRIPT plasmid (Stratagene), PSPO1 plasmid (Life Technologies), pcDNA2.1 plasmid (Invitrogen, Carlsbad CA), or pINCY plasmid (Incyte Genomics, Palo Alto CA). Recombinant plasmids were transformed into competent *E. coli* cells including XL1-Blue, XL1-BlueMRF, or SOLR from Stratagene or DH5 $\alpha$ , DH10B, or ElectroMAX DH10B from Life Technologies.

## II. Isolation of cDNA Clones

10 Plasmids obtained as described in Example I were recovered from host cells by in vivo excision using the UNIZAP vector system (Stratagene) or by cell lysis. Plasmids were purified using at least one of the following: a Magic or WIZARD Minipreps DNA purification system (Promega); an AGTC Miniprep purification kit (Edge Biosystems, Gaithersburg MD); and QIAWELL 8 Plasmid, QIAWELL 8 Plus Plasmid, QIAWELL 8 Ultra Plasmid purification systems or the R.E.A.L. PREP 96

15 plasmid purification kit from QIAGEN. Following precipitation, plasmids were resuspended in 0.1 ml of distilled water and stored, with or without lyophilization, at 4°C.

Alternatively, plasmid DNA was amplified from host cell lysates using direct link PCR in a high-throughput format (Rao, V.B. (1994) Anal. Biochem. 216:1-14). Host cell lysis and thermal cycling steps were carried out in a single reaction mixture. Samples were processed and stored in

20 384-well plates, and the concentration of amplified plasmid DNA was quantified fluorometrically using PICOGREEN dye (Molecular Probes, Eugene OR) and a FLUOROSKAN II fluorescence scanner (Labsystems Oy, Helsinki, Finland).

## III. Sequencing and Analysis

Incyte cDNA recovered in plasmids as described in Example II were sequenced as follows.

25 Sequencing reactions were processed using standard methods or high-throughput instrumentation such as the ABI CATALYST 800 (PE Biosystems) thermal cycler or the PTC-200 thermal cycler (MJ Research) in conjunction with the HYDRA microdispenser (Robbins Scientific) or the MICROLAB 2200 (Hamilton) liquid transfer system. cDNA sequencing reactions were prepared using reagents provided by Amersham Pharmacia Biotech or supplied in ABI sequencing kits such as the ABI

30 PRISM BIGDYE Terminator cycle sequencing ready reaction kit (PE Biosystems). Electrophoretic separation of cDNA sequencing reactions and detection of labeled polynucleotides were carried out using the MEGABACE 1000 DNA sequencing system (Molecular Dynamics); the ABI PRISM 373 or 377 sequencing system (PE Biosystems) in conjunction with standard ABI protocols and base calling software; or other sequence analysis systems known in the art. Reading frames within the

35 cDNA sequences were identified using standard methods (reviewed in Ausubel, 1997, supra, unit 7.7). Some of the cDNA sequences were selected for extension using the techniques disclosed in

#### Example VI.

The polynucleotide sequences derived from cDNA sequencing were assembled and analyzed using a combination of software programs which utilize algorithms well known to those skilled in the art. Table 5 summarizes the tools, programs, and algorithms used and provides applicable descriptions, references, and threshold parameters. The first column of Table 5 shows the tools, programs, and algorithms used, the second column provides brief descriptions thereof, the third column presents appropriate references, all of which are incorporated by reference herein in their entirety, and the fourth column presents, where applicable, the scores, probability values, and other parameters used to evaluate the strength of a match between two sequences (the higher the score, the greater the homology between two sequences). Sequences were analyzed using MACDNASIS PRO software (Hitachi Software Engineering, South San Francisco CA) and LASERGENE software (DNASTAR). Polynucleotide and polypeptide sequence alignments were generated using the default parameters specified by the clustal algorithm as incorporated into the MEGALIGN multisequence alignment program (DNASTAR), which also calculates the percent identity between aligned sequences.

The polynucleotide sequences were validated by removing vector, linker, and polyA sequences and by masking ambiguous bases, using algorithms and programs based on BLAST, dynamic programming, and dinucleotide nearest neighbor analysis. The sequences were then queried against a selection of public databases such as the GenBank primate, rodent, mammalian, vertebrate, and eukaryote databases, and BLOCKS, PRINTS, DOMO, PRODOM, and PFAM to acquire annotation using programs based on BLAST, FASTA, and BLIMPS. The sequences were assembled into full length polynucleotide sequences using programs based on Phred, Phrap, and Consed, and were screened for open reading frames using programs based on GeneMark, BLAST, and FASTA. The full length polynucleotide sequences were translated to derive the corresponding full length amino acid sequences, and these full length sequences were subsequently analyzed by querying against databases such as the GenBank databases (described above), SwissProt, BLOCKS, PRINTS, DOMO, PRODOM, Prosite, and Hidden Markov Model (HMM)-based protein family databases such as PFAM. HMM is a probabilistic approach which analyzes consensus primary structures of gene families. (See, e.g., Eddy, S.R. (1996) Curr. Opin. Struct. Biol. 6:361-365.)

The programs described above for the assembly and analysis of full length polynucleotide and amino acid sequences were also used to identify polynucleotide sequence fragments from SEQ ID NO:16-30. Fragments from about 20 to about 4000 nucleotides which are useful in hybridization and amplification technologies were described in The Invention section above.

#### IV. Analysis of Polynucleotide Expression

Northern analysis is a laboratory technique used to detect the presence of a transcript of a gene and involves the hybridization of a labeled nucleotide sequence to a membrane on which RNAs

from a particular cell type or tissue have been bound. (See, e.g., Sambrook, supra, ch. 7; Ausubel, 1995, supra, ch. 4 and 16.)

Analogous computer techniques applying BLAST were used to search for identical or related molecules in cDNA databases such as GenBank or LIFESEQ (Incyte Genomics). This analysis is much faster than multiple membrane-based hybridizations. In addition, the sensitivity of the computer search can be modified to determine whether any particular match is categorized as exact or similar. The basis of the search is the product score, which is defined as:

$$\frac{\text{BLAST Score} \times \text{Percent Identity}}{5 \times \text{minimum \{length(Seq. 1), length(Seq. 2)\}}$$

The product score takes into account both the degree of similarity between two sequences and the length of the sequence match. The product score is a normalized value between 0 and 100, and is calculated as follows: the BLAST score is multiplied by the percent nucleotide identity and the product is divided by (5 times the length of the shorter of the two sequences). The BLAST score is calculated by assigning a score of +5 for every base that matches in a high-scoring segment pair (HSP), and -4 for every mismatch. Two sequences may share more than one HSP (separated by gaps). If there is more than one HSP, then the pair with the highest BLAST score is used to calculate the product score. The product score represents a balance between fractional overlap and quality in a BLAST alignment. For example, a product score of 100 is produced only for 100% identity over the entire length of the shorter of the two sequences being compared. A product score of 70 is produced either by 100% identity and 70% overlap at one end, or by 88% identity and 100% overlap at the other. A product score of 50 is produced either by 100% identity and 50% overlap at one end, or 79% identity and 100% overlap.

The results of northern analyses are reported as a percentage distribution of libraries in which the transcript encoding SYNT occurred. Analysis involved the categorization of cDNA libraries by organ/tissue and disease. The organ/tissue categories included cardiovascular, dermatologic, developmental, endocrine, gastrointestinal, hematopoietic/immune, musculoskeletal, nervous, reproductive, and urologic. The disease/condition categories included cancer, inflammation, trauma, cell proliferation, neurological, and pooled. For each category, the number of libraries expressing the sequence of interest was counted and divided by the total number of libraries across all categories. Percentage values of tissue-specific and disease- or condition-specific expression are reported in Table 3.

#### V. Extension of SYNT Encoding Polynucleotides

The full length nucleic acid sequences of SEQ ID NO:16-30 were produced by extension of an appropriate fragment of the full length molecule using oligonucleotide primers designed from this fragment. One primer was synthesized to initiate 5' extension of the known fragment, and the other

primer, to initiate 3' extension of the known fragment. The initial primers were designed using OLIGO 4.06 software (National Biosciences), or another appropriate program, to be about 22 to 30 nucleotides in length, to have a GC content of about 50% or more, and to anneal to the target sequence at temperatures of about 68°C to about 72°C. Any stretch of nucleotides which would result in hairpin structures and primer-primer dimerizations was avoided.

Selected human cDNA libraries were used to extend the sequence. If more than one extension was necessary or desired, additional or nested sets of primers were designed.

High fidelity amplification was obtained by PCR using methods well known in the art. PCR was performed in 96-well plates using the PTC-200 thermal cycler (MJ Research, Inc.). The reaction mix contained DNA template, 200 nmol of each primer, reaction buffer containing  $Mg^{2+}$ ,  $(NH_4)_2SO_4$ , and  $\beta$ -mercaptoethanol, Taq DNA polymerase (Amersham Pharmacia Biotech), ELONGASE enzyme (Life Technologies), and Pfu DNA polymerase (Stratagene), with the following parameters for primer pair PCI A and PCI B: Step 1: 94°C, 3 min; Step 2: 94°C, 15 sec; Step 3: 60°C, 1 min; Step 4: 68°C, 2 min; Step 5: Steps 2, 3, and 4 repeated 20 times; Step 6: 68°C, 5 min; Step 7: storage at 4°C. In the alternative, the parameters for primer pair T7 and SK+ were as follows: Step 1: 94°C, 3 min; Step 2: 94°C, 15 sec; Step 3: 57°C, 1 min; Step 4: 68°C, 2 min; Step 5: Steps 2, 3, and 4 repeated 20 times; Step 6: 68°C, 5 min; Step 7: storage at 4°C.

The concentration of DNA in each well was determined by dispensing 100  $\mu$ l PICOGREEN quantitation reagent (0.25% (v/v) PICOGREEN; Molecular Probes, Eugene OR) dissolved in 1X TE and 0.5  $\mu$ l of undiluted PCR product into each well of an opaque fluorimeter plate (Corning Costar, Acton MA), allowing the DNA to bind to the reagent. The plate was scanned in a Fluoroskan II (Labsystems Oy, Helsinki, Finland) to measure the fluorescence of the sample and to quantify the concentration of DNA. A 5  $\mu$ l to 10  $\mu$ l aliquot of the reaction mixture was analyzed by electrophoresis on a 1 % agarose mini-gel to determine which reactions were successful in extending the sequence.

The extended nucleotides were desalted and concentrated, transferred to 384-well plates, digested with CviJI cholera virus endonuclease (Molecular Biology Research, Madison WI), and sonicated or sheared prior to religation into pUC 18 vector (Amersham Pharmacia Biotech). For shotgun sequencing, the digested nucleotides were separated on low concentration (0.6 to 0.8%) agarose gels, fragments were excised, and agar digested with Agar ACE (Promega). Extended clones were religated using T4 ligase (New England Biolabs, Beverly MA) into pUC 18 vector (Amersham Pharmacia Biotech), treated with Pfu DNA polymerase (Stratagene) to fill-in restriction site overhangs, and transfected into competent *E. coli* cells. Transformed cells were selected on antibiotic-containing media, and individual colonies were picked and cultured overnight at 37°C in 384-well plates in LB/2x carb liquid media.

The cells were lysed, and DNA was amplified by PCR using Taq DNA polymerase

(Amersham Pharmacia Biotech) and Pfu DNA polymerase (Stratagene) with the following parameters: Step 1: 94°C, 3 min; Step 2: 94°C, 15 sec; Step 3: 60°C, 1 min; Step 4: 72°C, 2 min; Step 5: steps 2, 3, and 4 repeated 29 times; Step 6: 72°C, 5 min; Step 7: storage at 4°C. DNA was quantified by PICOGREEN reagent (Molecular Probes) as described above. Samples with low DNA recoveries were reamplified using the same conditions as described above. Samples were diluted with 20% dimethylsulfoxide (1:2, v/v), and sequenced using DYENAMIC energy transfer sequencing primers and the DYENAMIC DIRECT kit (Amersham Pharmacia Biotech) or the ABI PRISM BIGDYE Terminator cycle sequencing ready reaction kit (PE Biosystems).

In like manner, the polynucleotide sequences of SEQ ID NO:16-30 are used to obtain 5' regulatory sequences using the procedure above, along with oligonucleotides designed for such extension, and an appropriate genomic library.

#### V. Chromosomal Mapping of SNYT Encoding Polynucleotides

The cDNA sequences which were used to assemble SEQ ID NO:16-30 were compared with sequences from the Incyte LIFESEQ database and public domain databases using BLAST and other implementations of the Smith-Waterman algorithm. Sequences from these databases that matched SEQ ID NO:16-30 were assembled into clusters of contiguous and overlapping sequences using assembly algorithms such as Phrap (Table 5). Radiation hybrid and genetic mapping data available from public resources such as the Stanford Human Genome Center (SHGC), Whitehead Institute for Genome Research (WIGR), and Généthon were used to determine if any of the clustered sequences had been previously mapped. Inclusion of a mapped sequence in a cluster resulted in the assignment of all sequences of that cluster, including its particular SEQ ID NO., to that map location.

The genetic map locations of SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:21, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, and SEQ ID NO:30 are described in The Invention as ranges, or intervals, of human chromosomes. More than one map location is reported for SEQ ID NO:28 and SEQ ID NO:29, indicating that previously mapped sequences having similarity, but not complete identity, to SEQ ID NO:28 and SEQ ID NO:29 were assembled into their respective clusters. The map position of an interval, in centiMorgans, is measured relative to the terminus of the chromosome's p-arm. (The centiMorgan (cM) is a unit of measurement based on recombination frequencies between chromosomal markers. On average, 1 cM is roughly equivalent to 1 megabase (Mb) of DNA in humans, although this can vary widely due to hot and cold spots of recombination.) The cM distances are based on genetic markers mapped by Généthon which provide boundaries for radiation hybrid markers whose sequences were included in each of the clusters. Diseases associated with the public and Incyte sequences located within the indicated intervals are also reported in the Invention where applicable. Human genome maps and other resources available to the public, such as the NCBI "GeneMap'99" World Wide Web site which can be accessed at <http://www.ncbi.nlm.nih.gov/genemap>, can be employed to determine if



previously identified disease genes map within or in proximity to the intervals indicated above.

#### **VI. Labeling and Use of Individual Hybridization Probes**

Hybridization probes derived from SEQ ID NO:16-30 are employed to screen cDNAs, genomic DNAs, or mRNAs. Although the labeling of oligonucleotides, consisting of about 20 base pairs, is specifically described, essentially the same procedure is used with larger nucleotide fragments. Oligonucleotides are designed using state-of-the-art software such as OLIGO 4.06 software (National Biosciences) and labeled by combining 50 pmol of each oligomer, 250  $\mu$ Ci of [ $\gamma$ - $^{32}$ P] adenosine triphosphate (Amersham Pharmacia Biotech), and T4 polynucleotide kinase (DuPont NEN, Boston MA). The labeled oligonucleotides are substantially purified using a SEPHADEX G-25 superfine size exclusion dextran bead column (Amersham Pharmacia Biotech). An aliquot containing  $10^7$  counts per minute of the labeled probe is used in a typical membrane-based hybridization analysis of human genomic DNA digested with one of the following endonucleases: Ase I, Bgl II, Eco RI, Pst I, Xba I, or Pvu II (DuPont NEN).

The DNA from each digest is fractionated on a 0.7% agarose gel and transferred to nylon membranes (Nytran Plus, Schleicher & Schuell, Durham NH). Hybridization is carried out for 16 hours at 40°C. To remove nonspecific signals, blots are sequentially washed at room temperature under conditions of up to, for example, 0.1 x saline sodium citrate and 0.5% sodium dodecyl sulfate. Hybridization patterns are visualized using autoradiography or an alternative imaging means and compared.

#### **VII. Microarrays**

The linkage or synthesis of array elements upon a microarray can be achieved utilizing photolithography, piezoelectric printing (ink-jet printing, See, e.g., Baldeschweiler, supra), mechanical microspotting technologies, and derivatives thereof. The substrate in each of the aforementioned technologies should be uniform and solid with a non-porous surface (Skena (1999), supra). Suggested substrates include silicon, silica, glass slides, glass chips, and silicon wafers. Alternatively, a procedure analogous to a dot or slot blot may also be used to arrange and link elements to the surface of a substrate using thermal, UV, chemical, or mechanical bonding procedures. A typical array may be produced using available methods and machines well known to those of ordinary skill in the art and may contain any appropriate number of elements. (See, e.g., Schena, M. et al. (1995) Science 270:467-470; Shalon, D. et al. (1996) Genome Res. 6:639-645; Marshall, A. and J. Hodgson (1998) Nat. Biotechnol. 16:27-31.)

Full length cDNAs, Expressed Sequence Tags (ESTs), or fragments or oligomers thereof may comprise the elements of the microarray. Fragments or oligomers suitable for hybridization can be selected using software well known in the art such as LASERGENE software (DNASTAR). The array elements are hybridized with polynucleotides in a biological sample. The polynucleotides in the biological sample are conjugated to a fluorescent label or other molecular tag for ease of detection.

After hybridization, nonhybridized nucleotides from the biological sample are removed, and a fluorescence scanner is used to detect hybridization at each array element. Alternatively, laser desorption and mass spectrometry may be used for detection of hybridization. The degree of complementarity and the relative abundance of each polynucleotide which hybridizes to an element on the microarray may be assessed. In one embodiment, microarray preparation and usage is described in detail below.

#### Tissue or Cell Sample Preparation

Total RNA is isolated from tissue samples using the guanidinium thiocyanate method and poly(A)<sup>+</sup> RNA is purified using the oligo-(dT) cellulose method. Each poly(A)<sup>+</sup> RNA sample is reverse transcribed using MMLV reverse-transcriptase, 0.05 pg/ $\mu$ l oligo-(dT) primer (21mer), 1X first strand buffer, 0.03 units/ $\mu$ l RNase inhibitor, 500  $\mu$ M dATP, 500  $\mu$ M dGTP, 500  $\mu$ M dTTP, 40  $\mu$ M dCTP, 40  $\mu$ M dCTP-Cy3 (BDS) or dCTP-Cy5 (Amersham Pharmacia Biotech). The reverse transcription reaction is performed in a 25 ml volume containing 200 ng poly(A)<sup>+</sup> RNA with GEMBRIGHT kits (Incyte). Specific control poly(A)<sup>+</sup> RNAs are synthesized by *in vitro* transcription from non-coding yeast genomic DNA. After incubation at 37 °C for 2 hr, each reaction sample (one with Cy3 and another with Cy5 labeling) is treated with 2.5 ml of 0.5M sodium hydroxide and incubated for 20 minutes at 85 °C to stop the reaction and degrade the RNA. Samples are purified using two successive CHROMA SPIN 30 gel filtration spin columns (CLONTECH Laboratories, Inc. (CLONTECH), Palo Alto CA) and after combining, both reaction samples are ethanol precipitated using 1 ml of glycogen (1 mg/ml), 60 ml sodium acetate, and 300 ml of 100% ethanol. The sample is then dried to completion using a SpeedVAC (Savant Instruments Inc., Holbrook NY) and resuspended in 14  $\mu$ l 5X SSC/0.2% SDS.

#### Microarray Preparation

Sequences of the present invention are used to generate array elements. Each array element is amplified from bacterial cells containing vectors with cloned cDNA inserts. PCR amplification uses primers complementary to the vector sequences flanking the cDNA insert. Array elements are amplified in thirty cycles of PCR from an initial quantity of 1-2 ng to a final quantity greater than 5  $\mu$ g. Amplified array elements are then purified using SEPHACRYL-400 (Amersham Pharmacia Biotech).

Purified array elements are immobilized on polymer-coated glass slides. Glass microscope slides (Corning) are cleaned by ultrasound in 0.1% SDS and acetone, with extensive distilled water washes between and after treatments. Glass slides are etched in 4% hydrofluoric acid (VWR Scientific Products Corporation (VWR), West Chester PA), washed extensively in distilled water, and coated with 0.05% aminopropyl silane (Sigma) in 95% ethanol. Coated slides are cured in a 110°C oven.

Array elements are applied to the coated glass substrate using a procedure described in US

Patent No. 5,807,522, incorporated herein by reference. 1  $\mu$ l of the array element DNA, at an average concentration of 100 ng/ $\mu$ l, is loaded into the open capillary printing element by a high-speed robotic apparatus. The apparatus then deposits about 5 nl of array element sample per slide.

Microarrays are UV-crosslinked using a STRATALINKER UV-crosslinker (Stratagene).

- 5 Microarrays are washed at room temperature once in 0.2% SDS and three times in distilled water. Non-specific binding sites are blocked by incubation of microarrays in 0.2% casein in phosphate buffered saline (PBS) (Tropix, Inc., Bedford MA) for 30 minutes at 60 °C followed by washes in 0.2% SDS and distilled water as before.

#### Hybridization

- 10 Hybridization reactions contain 9  $\mu$ l of sample mixture consisting of 0.2  $\mu$ g each of Cy3 and Cy5 labeled cDNA synthesis products in 5X SSC, 0.2% SDS hybridization buffer. The sample mixture is heated to 65 °C for 5 minutes and is aliquoted onto the microarray surface and covered with an 1.8 cm<sup>2</sup> coverslip. The arrays are transferred to a waterproof chamber having a cavity just slightly larger than a microscope slide. The chamber is kept at 100% humidity internally by the  
15 addition of 140  $\mu$ l of 5X SSC in a corner of the chamber. The chamber containing the arrays is incubated for about 6.5 hours at 60 °C. The arrays are washed for 10 min at 45 °C in a first wash buffer (1X SSC, 0.1% SDS), three times for 10 minutes each at 45 °C in a second wash buffer (0.1X SSC), and dried.

#### Detection

- 20 Reporter-labeled hybridization complexes are detected with a microscope equipped with an Innova 70 mixed gas 10 W laser (Coherent, Inc., Santa Clara CA) capable of generating spectral lines at 488 nm for excitation of Cy3 and at 632 nm for excitation of Cy5. The excitation laser light is focused on the array using a 20X microscope objective (Nikon, Inc., Melville NY). The slide containing the array is placed on a computer-controlled X-Y stage on the microscope and raster-  
25 scanned past the objective. The 1.8 cm x 1.8 cm array used in the present example is scanned with a resolution of 20 micrometers.

- In two separate scans, a mixed gas multiline laser excites the two fluorophores sequentially. Emitted light is split, based on wavelength, into two photomultiplier tube detectors (PMT R1477, Hamamatsu Photonics Systems, Bridgewater NJ) corresponding to the two fluorophores. Appropriate  
30 filters positioned between the array and the photomultiplier tubes are used to filter the signals. The emission maxima of the fluorophores used are 565 nm for Cy3 and 650 nm for Cy5. Each array is typically scanned twice, one scan per fluorophore using the appropriate filters at the laser source, although the apparatus is capable of recording the spectra from both fluorophores simultaneously.

- The sensitivity of the scans is typically calibrated using the signal intensity generated by a  
35 cDNA control species added to the sample mixture at a known concentration. A specific location on the array contains a complementary DNA sequence, allowing the intensity of the signal at that

location to be correlated with a weight ratio of hybridizing species of 1:100,000. When two samples from different sources (e.g., representing test and control cells), each labeled with a different fluorophore, are hybridized to a single array for the purpose of identifying genes that are differentially expressed, the calibration is done by labeling samples of the calibrating cDNA with the two fluorophores and adding identical amounts of each to the hybridization mixture.

The output of the photomultiplier tube is digitized using a 12-bit RTI-835H analog-to-digital (A/D) conversion board (Analog Devices, Inc., Norwood MA) installed in an IBM-compatible PC computer. The digitized data are displayed as an image where the signal intensity is mapped using a linear 20-color transformation to a pseudocolor scale ranging from blue (low signal) to red (high signal). The data is also analyzed quantitatively. Where two different fluorophores are excited and measured simultaneously, the data are first corrected for optical crosstalk (due to overlapping emission spectra) between the fluorophores using each fluorophore's emission spectrum.

A grid is superimposed over the fluorescence signal image such that the signal from each spot is centered in each element of the grid. The fluorescence signal within each element is then integrated to obtain a numerical value corresponding to the average intensity of the signal. The software used for signal analysis is the GEMTOOLS gene expression analysis program (Incyte).

### VIII. Complementary Polynucleotides

Sequences complementary to the SYNT-encoding sequences, or any parts thereof, are used to detect, decrease, or inhibit expression of naturally occurring SYNT. Although use of oligonucleotides comprising from about 15 to 30 base pairs is described, essentially the same procedure is used with smaller or with larger sequence fragments. Appropriate oligonucleotides are designed using OLIGO 4.06 software (National Biosciences) and the coding sequence of SYNT. To inhibit transcription, a complementary oligonucleotide is designed from the most unique 5' sequence and used to prevent promoter binding to the coding sequence. To inhibit translation, a complementary oligonucleotide is designed to prevent ribosomal binding to the SYNT-encoding transcript.

### IX. Expression of SYNT

Expression and purification of SYNT is achieved using bacterial or virus-based expression systems. For expression of SYNT in bacteria, cDNA is subcloned into an appropriate vector containing an antibiotic resistance gene and an inducible promoter that directs high levels of cDNA transcription. Examples of such promoters include, but are not limited to, the *trp-lac* (*tac*) hybrid promoter and the T5 or T7 bacteriophage promoter in conjunction with the *lac* operator regulatory element. Recombinant vectors are transformed into suitable bacterial hosts, e.g., BL21(DE3). Antibiotic resistant bacteria express SYNT upon induction with isopropyl beta-D-thiogalactopyranoside (IPTG). Expression of SYNT in eukaryotic cells is achieved by infecting insect or mammalian cell lines with recombinant Autographica californica nuclear polyhedrosis virus

(AcMNPV), commonly known as baculovirus. The nonessential polyhedrin gene of baculovirus is replaced with cDNA encoding SYNT by either homologous recombination or bacterial-mediated transposition involving transfer plasmid intermediates. Viral infectivity is maintained and the strong polyhedrin promoter drives high levels of cDNA transcription. Recombinant baculovirus is used to infect *Spodoptera frugiperda* (Sf9) insect cells in most cases, or human hepatocytes, in some cases. Infection of the latter requires additional genetic modifications to baculovirus. (See Engelhard, E.K. et al. (1994) Proc. Natl. Acad. Sci. USA 91:3224-3227; Sandig, V. et al. (1996) Hum. Gene Ther. 7:1937-1945.)

In most expression systems, SYNT is synthesized as a fusion protein with, e.g., glutathione S-transferase (GST) or a peptide epitope tag, such as FLAG or 6-His, permitting rapid, single-step, affinity-based purification of recombinant fusion protein from crude cell lysates. GST, a 26-kilodalton enzyme from *Schistosoma japonicum*, enables the purification of fusion proteins on immobilized glutathione under conditions that maintain protein activity and antigenicity (Amersham Pharmacia Biotech). Following purification, the GST moiety can be proteolytically cleaved from SYNT at specifically engineered sites. FLAG, an 8-amino acid peptide, enables immunoaffinity purification using commercially available monoclonal and polyclonal anti-FLAG antibodies (Eastman Kodak). 6-His, a stretch of six consecutive histidine residues, enables purification on metal-chelate resins (QIAGEN). Methods for protein expression and purification are discussed in Ausubel (1995, *supra*, ch. 10 and 16). Purified SYNT obtained by these methods can be used directly in the assays shown in Examples X and XIV.

#### X. Demonstration of SYNT Activity

An SYNT activity assay measures aminoacylation of tRNA in the presence of a radiolabeled substrate. A cell-free extract depleted of endogenous aminoacyl-tRNA synthetase is prepared from *Escherichia coli*. SYNT, either biochemically purified or recombinantly produced, is added to the cell free extract. The cell-free extract is incubated with [<sup>14</sup>C]-labeled amino acid under conditions favorable for translation. Incorporation of the [<sup>14</sup>C]-labeled amino acid into acid-precipitable aminoacyl-tRNA is measured using a radioisotope counter. The amount of the [<sup>14</sup>C]-labeled amino acid incorporated into aminoacyl tRNA is proportional to the amount of SYNT activity. (See, for example, Ibba, M. et al. (1997) Science 278:1119-1122).

Alternatively, SYNT activity may be assayed as follows. SYNT, or biologically active fragments thereof, are labeled with <sup>125</sup>I Bolton-Hunter reagent. (See, e.g., Bolton et al. (1973) Biochem. J. 133:529.) Candidate molecules previously arrayed in the wells of a multi-well plate are incubated with the labeled SYNT, washed, and any wells with labeled SYNT complex are assayed. Data obtained using different concentrations of SYNT are used to calculate values for the number, affinity, and association of SYNT with the candidate molecules.

#### XI. Functional Assays

SYNT function is assessed by expressing the sequences encoding SYNT at physiologically elevated levels in mammalian cell culture systems. cDNA is subcloned into a mammalian expression vector containing a strong promoter that drives high levels of cDNA expression. Vectors of choice include pCMV SPORT plasmid (Life Technologies) and pCR3.1 plasmid (Invitrogen), both of which contain the cytomegalovirus promoter. 5-10  $\mu$ g of recombinant vector are transiently transfected into a human cell line, for example, an endothelial or hematopoietic cell line, using either liposome formulations or electroporation. 1-2  $\mu$ g of an additional plasmid containing sequences encoding a marker protein are co-transfected. Expression of a marker protein provides a means to distinguish transfected cells from nontransfected cells and is a reliable predictor of cDNA expression from the recombinant vector. Marker proteins of choice include, e.g., Green Fluorescent Protein (GFP; Clontech), CD64, or a CD64-GFP fusion protein. Flow cytometry (FCM), an automated, laser optics-based technique, is used to identify transfected cells expressing GFP or CD64-GFP and to evaluate the apoptotic state of the cells and other cellular properties. FCM detects and quantifies the uptake of fluorescent molecules that diagnose events preceding or coincident with cell death. These events include changes in nuclear DNA content as measured by staining of DNA with propidium iodide; changes in cell size and granularity as measured by forward light scatter and 90 degree side light scatter; down-regulation of DNA synthesis as measured by decrease in bromodeoxyuridine uptake; alterations in expression of cell surface and intracellular proteins as measured by reactivity with specific antibodies; and alterations in plasma membrane composition as measured by the binding of fluorescein-conjugated Annexin V protein to the cell surface. Methods in flow cytometry are discussed in Ormerod, M.G. (1994) Flow Cytometry, Oxford, New York NY.

The influence of SYNT on gene expression can be assessed using highly purified populations of cells transfected with sequences encoding SYNT and either CD64 or CD64-GFP. CD64 and CD64-GFP are expressed on the surface of transfected cells and bind to conserved regions of human immunoglobulin G (IgG). Transfected cells are efficiently separated from nontransfected cells using magnetic beads coated with either human IgG or antibody against CD64 (DYNAL, Lake Success NY). mRNA can be purified from the cells using methods well known by those of skill in the art. Expression of mRNA encoding SYNT and other genes of interest can be analyzed by northern analysis or microarray techniques.

## **XII. Production of SYNT Specific Antibodies**

SYNT substantially purified using polyacrylamide gel electrophoresis (PAGE; see, e.g., Harrington, M.G. (1990) *Methods Enzymol.* 182:488-495), or other purification techniques, is used to immunize rabbits and to produce antibodies using standard protocols.

Alternatively, the SYNT amino acid sequence is analyzed using LASERGENE software (DNASTAR) to determine regions of high immunogenicity, and a corresponding oligopeptide is synthesized and used to raise antibodies by means known to those of skill in the art. Methods for

selection of appropriate epitopes, such as those near the C-terminus or in hydrophilic regions are well described in the art. (See, e.g., Ausubel, 1995, supra, ch. 11.)

Typically, oligopeptides of about 15 residues in length are synthesized using an ABI 431A peptide synthesizer (PE Biosystems) using Fmoc chemistry and coupled to KLH (Sigma-Aldrich, St. Louis MO) by reaction with N-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS) to increase immunogenicity. (See, e.g., Ausubel, 1995, supra.) Rabbits are immunized with the oligopeptide-KLH complex in complete Freund's adjuvant. Resulting antisera are tested for antipeptide and anti-SYNT activity by, for example, binding the peptide or SYNT to a substrate, blocking with 1% BSA, reacting with rabbit antisera, washing, and reacting with radio-iodinated goat anti-rabbit IgG.

#### 10 **XIII. Purification of Naturally Occurring SYNT Using Specific Antibodies**

Naturally occurring or recombinant SYNT is substantially purified by immunoaffinity chromatography using antibodies specific for SYNT. An immunoaffinity column is constructed by covalently coupling anti-SYNT antibody to an activated chromatographic resin, such as CNBr-activated SEPHAROSE (Amersham Pharmacia Biotech). After the coupling, the resin is  
15 blocked and washed according to the manufacturer's instructions.

Media containing SYNT are passed over the immunoaffinity column, and the column is washed under conditions that allow the preferential absorbance of SYNT (e.g., high ionic strength buffers in the presence of detergent). The column is eluted under conditions that disrupt antibody/SYNT binding (e.g., a buffer of pH 2 to pH 3, or a high concentration of a chaotrope, such as urea or thiocyanate ion), and SYNT is collected.  
20

#### **XIV. Identification of Molecules Which Interact with SYNT**

SYNT, or biologically active fragments thereof, are labeled with <sup>125</sup>I Bolton-Hunter reagent. (See, e.g., Bolton A.E. and W.M. Hunter (1973) Biochem. J. 133:529-539.) Candidate molecules previously arrayed in the wells of a multi-well plate are incubated with the labeled SYNT, washed,  
25 and any wells with labeled SYNT complex are assayed. Data obtained using different concentrations of SYNT are used to calculate values for the number, affinity, and association of SYNT with the candidate molecules.

Alternatively, molecules interacting with SYNT are analyzed using the yeast two-hybrid system as described in Fields, S. and O. Song (1989, Nature 340:245-246), or using commercially  
30 available kits based on the two-hybrid system, such as the MATCHMAKER system (Clontech).

SYNT may also be used in the PATHCALLING process (CuraGen Corp., New Haven CT) which employs the yeast two-hybrid system in a high-throughput manner to determine all interactions between the proteins encoded by two large libraries of genes (Nandabalan, K. et al. (2000) U.S. Patent No. 6,057,101).

35

Various modifications and variations of the described methods and systems of the invention

will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with certain embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention  
5 which are obvious to those skilled in molecular biology or related fields are intended to be within the scope of the following claims.



Table I

Polypeptide SEQ ID NO:	Nucleotide SEQ ID NO:	Clone ID	Library	Fragments
1	16	1806212	SINTNOT13	268731R1 (HNT2NOT01), 410547R1 (BRSTNOT01), 642383F1 (BRSTNOT03), 642383R6 (BRSTNOT03), 1484778F6 (CORFNOT02), 1577276X13 (LNODNOT03), 1577276X14 (LNODNOT03), 1577276X16 (LNODNOT03), 1733808F6 (BRSTTUT08), 2057185R6 (BEPINOT01)
2	17	2083883	UTRSNOT08	1308705F1 (COLNFET02), 1337653F1 (COLNNOT13), 1430326F1 (SINTBST01), 1525049F6 (UCMCL5T01), 1525049T7 (UCMCL5T01), 1807032F6 (SINTNOT13), 2083883X31C1 (UTRSNOT08), 20838831X54C1, SAEA01919R1, SAEA00070F1
3	18	2454288	ENDANOT01	1334395F6 (COLNNOT13), 1607276F6 (LUNGNOT15), 1930074F6 (COLNTUT03), 1978977R6 (LUNGTUT03), 2054860R6 (BEPINOT01), 2454288H1 (ENDANOT01), 2791177H2 (COLNTUT16), 4049241H1 (SINTNOT18), 4787755H1 (BRATNOT03)
4	19	1513539	PANCTUT01	285705F1 (EOSIHET02), 515506F1 (MMLRIDT01), 515506R1 (MMLRIDT01), 723201R1 (SYNOOAT01), 726178R1 (SYNOOAT01), 906091R1 (COLNNOT08), 1492309T1 (PROSNON01), 1513539H1 (PANCTUT01), 2632033H1 (COLNTUT15)
5	20	2148623	BRAINOT09	655968H1 (EOSINOT03), 820407T1 (KERANOT02), 1383948F1 (BRAITUT08), 1383996T6 (BRAITUT08), 1441048R1 (THYRNOT03), 1579330F6 (DUODNOT01), 2148623H1 (BRAINOT09), 3170589H1 (BRSTNOT18), 3251591H1 (SEMVNOT03), 3618285H1 (EPIPNOT01), 3637486H1 (LUNGNOT30), g1524807
6	21	2579405	KIDNTUT13	2457R1 (U9337NOT01), 270182R1 (HNT2NOT01), 730115H1 (LUNGNOT03), 991450H1 (COLNNOT11), 994055T1 (COLNNOT11), 1308496H1 (COLNFET02), 1808269F6 (SINTNOT13), 1875536T6 (LEUKNOT02), 1978055H1 (LUNGTUT03), 2579405H1 (KIDNTUT13), 2583944H1 (BRAITUT22)
7	22	2662427	ADRENOT08	283147F1 (CARDNOT01), 904594R2 (COLNNOT07), 2662427H1 (ADRENOT08), 2860066H1 (SININOT03), 3078653H1 (BRAIUNT01), 3852430F6 (BRAITUT12), 3852430T6 (BRAITUT12), SCDA09024V1, SCDA05143V1

Table 1 (cont.)

Polypeptide SEQ ID NO:	Nucleotide SEQ ID NO:	Clone ID	Library	Fragments
8	23	2844928	DRGLNOT01	1466601F1 (FANCTUT02), 1662986F6 (BRSTNOT09), 1732880F6 (BRSTTUT08), 1804107F6 (SINTNOT13), 2521851F6 (BRAITUT21), 2662837X303B1 (ADRENOT08), 2844928H1 (DRGLNOT01), 2967532H1 (SCORNOT04), 3774082H1 (BRSTNOT25), 5871970H1 (COLTDIT04)
9	24	3231586	COTRNOT01	319277H1 (EOSIHET02), 920940H1 (PATRNOT02), 1445995H1 (PLACNOT02), 1517438F1 (FANCTUT01), 1963042H1 (BRSTNOT04), 2023159H1 (CONNOT01), 2932203H1 (UTRSNOT01), 3231586H1 (COTRNOT01), 3346128H1 (BRAITUT24), g1474393
10	25	3580770	293TF3T01	269986R1 (HNT2NOT01), 269986R6 (HNT2NOT01), 3580770H1 (293TF3T01), 3602575F6 (DRGTNOT01), 3602575T6 (DRGTNOT01), g1839725
11	26	3778612	BRSTNOT27	3778612F6 (BRSTNOT27), 3778612H1 (BRSTNOT27), 4063060T6 (BRAINOT21)
12	27	4574912	PROSTMT02	224714F1 (PANCNOT01), 452757F1 (TLYMNOT02), 1210705R7 (BRSTNOT02), 1437821F6 (PANCNOT08), 2700694F6 (OVARUTUT10), 3963634H1 (PROSNOT14), 3964731H1 (PROSNOT14), 4574912H1 (PROSTMT02), 5035739H1 (LIVRTUT13)
13	28	5630806	PLACFER01	1649584X20C1 (PROSTUT09), 1649855X12C1 (PROSTUT09), 1650519X14C1 (PROSTUT09), 2054061R6 (BEPINOT01), 2347805X304V1 (COLSUCT01), 3384595H1 (ESOGNOT04), 5630806H1 (PLACFER01)
14	29	5854855	FIBAUNT02	746434R1 (BPAITUT01), 815240R1 (OVARUTUT01), 2515988F6 (LIVRTUT04), 3105953F6 (BRSTTUT15), 5854855H1 (FIBAUNT02)
15	30	5993973	FTUBTUT02	270363H1 (HNT2NOT01), 1863382F6 (PROSNOT19), 2696339F6 (UTRSNOT12), 3500696H1 (PROSTUT13), 4247086H1 (BRABDIT01), 5195020H1 (LUNLTUT04), 5591818H1 (COLCDIT03), 6023466H1 (TESTNOT11)

Table 2

Polypeptide SEQ ID NO:	Amino Acid Residues	Potential Phosphorylation Sites	Potential Glycosyla- tion Sites	Signature Sequences, Motifs, and Domains	Homologous Sequences	Analytical Methods and Databases
1	1176	T7 T238 T543 S35 S68 S167 S175 T224 S241 T379 S396 T444 S453 S518 T552 S593 S673 T749 T769 T791 S978 S1042 T1083 T26 T41 S128 S153 T293 T327 S368 T557 S697 S714 S783 T925 S1082 T1136 S1153 Y111 Y666	N33 N333 N439 N882	Leucine-tRNA ligase domain T303-I870 Amino-transfer RNA synthetase N36-F88 N713-N723	similar to leucyl-tRNA synthetase [Arabidopsis thaliana] g2160156	MOTIFS BLAST-GenBank BLAST-DOMO PROFILESCAN BLAST-PRODOM BLIMPS-BLOCKS
2	739	S28 T132 S185 S282 S288 S307 T320 T352 S451 S661 T728 S732 S116 S163 S168 S261 T398 S436 T590 S633 T714 Y173 Y349 Y725 Y734	N102 N588 N619	AMP-binding domain S163-V629, N294-V345 E309-I320, T321-I329 Long-chain acyl-CoA synthetase P158-E280	fatty acid coenzyme A ligase 5 [Homo sapiens] g6174680	MOTIFS BLAST-GenBank BLAST-DOMO PROFILESCAN BLAST-PRODOM BLIMPS-BLOCKS HMMER-PFAM BLIMPS-PRINTS
3	589	S179 T18 T20 S40 S138 T193 T256 T298 T354 S419 S485 T570 S579 S5 T114 T174	N187	Phenylalanyl-tRNA synthetase P2-Q556, N293-L589 Synthetase b chain D59-L84, R355-Y374 I439-N465	phenylalanyl tRNA synthetase beta subunit [M. musculus] g4633656	MOTIFS BLAST-GenBank BLAST-PRODOM BLIMPS-PRODOM

Table 2 (cont.)

Polypeptide SEQ ID NO:	Amino Acid Residues	Potential Phosphorylation Sites	Potential Glycosyla- tion Sites	Signature Sequences, Motifs, and Domains	Homologous Sequences	Analytical Methods and Databases
4	157	S26 S69 T131 T132 S108	N18	Signal peptide: M1-V66 Acetyl-CoA carboxylase carboxyl transferase alpha subunit signature: G128-I139	acetyl- coenzyme A synthetase [Penicillium chrysogenum] g265229	BLAST-GenBank SPSCAN BLOCKS-PRINTS MOTIFS
5	643	T308 T337 S402 S433 T454 S34 S101 T408 S530 S621	N9 N33 N90 N104 N259 N306 N431 N571 N634	Class II glutamine amidotransferase motif: L27-K36, G545-F560 (p<0.01) Transferase/ligase motif: R30-A224, S258-F356, P404-Q484	putative asparagine synthase [S. pombe] g3560144	BLAST GenBank BLIMPS-BLOCKS BLAST-PRODOR MOTIFS
6	660	T282 S315 S329 T487 T596 S9 S276 T459 T624 Y230	N140	Class-I aminocacyl tRNA synthetase signature: I74-M660, V183-L233, P201-L212, S519 N529 Arg-tRNA synthetase: K193-L224, V232-F245, T374-R395, H237-M660, S78-M660	arginyl-tRNA synthetase, ArgRS [Homo sapiens] g1217668	BLAST-GenBank HMMER-PFAM BLIMPS-BLOCKS PROFILERSCAN BLIMPS-PFAM BLAST-PRODOR BLAST-DMO MOTIFS

Table 2 (cont.)

Polypeptide SEQ ID NO:	Amino Acid Residues	Potential Phosphorylation Sites	Potential Glycosyla- tion Sites	Signature Sequences, Motifs, and Domains	Homologous Sequences	Analytical Methods and Databases
7	725	S723 S80 S220 S238 T351 S371 T414 T465 S591 S641 T38 T45 T67 T165 S230 T252 T407 S413 S454 S499 S541 T554 T685 S688 T693 S719	N48 N472 N546 N552	Biotin-requiring enzyme signature: P652-F714, I657-R707 Biotin attachment site: K681 Acetyl-CoA biotin carboxyl carrier protein signature: F661-M675, V676-P689 Carbamoyl-PO4 synthase signature: K51-P490, E193-K494, F333-L340, L245-P290, Y270-K494, F331-G365	3-methyl- crotonyl CoA carboxylase homolog [C. elegans] g3876562	BLAST-GenBank HMMER-PFAM BLIMPS-BLOCKS PROFILESCAN BLIMPS-PRINTS BLAST-PRODOM BLAST-DOMO MOTIFS
8	644	S30 S253 S370 T457 T496 T593 T594 S43 T65 S207 S237 S269 S626 Y247 Y521	N282	AMP-binding domain signature: E79-V538, T101-V540, S240-L293, H255-L275, F262-H277, I260-K271, V570-R635 Ligase/synthetase signature: T356-R640	predicted long chain fatty acid CoA ligase [C. elegans] g4262587	BLAST-GenBank HMMER-PFAM BLIMPS-BLOCKS PROFILESCAN BLIMPS-PRINTS BLAST-PRODOM BLAST-DOMO MOTIFS
9	504	S122 S134 S428 S111 T349	N9 N39 N209 N426	Signal peptide: M1-A67 Aminotransferase class- II pyridoxal-PO4 attachment site: A104-R113		SPSCAN BLIMPS-BLOCKS MOTIFS

Table 2 (cont.)

Polypeptide SEQ ID NO:	Amino Acid Residues	Potential Phosphorylation Sites	Potential Glycosyla- tion Sites	Signature Sequences, Motifs, and Domains	Homologous Sequences	Analytical Methods and Databases
10	489	S51 T150 T220 S465 S477 T90 S200 T250 T350 S433 S467 Y83		RNA methyl transferase SpoU family: P344-G354 CTP synthetase domain: K2-N455, M1-Y457, M335-Y457	CTP synthetase homolog (CTPsh) [Mus musculus] g1654185	BLAST-GenBank BLIMPS-PFAM BLAST-PRODOM BLAST-DOMO MOTIFS
11	258	S105 S115 S139		Signal peptide: M1-G43 ATP synthase signature: Y98-L129 S-adenosyl methionine synthetase signature: A209-V257		SPSCAN HMMER-PFAM PROFILES SCAN MOTIFS
12	555	S22 T72 T110 S132 S165 S190 S258 S342 S363 S446 S539 S31 T179 S212 S312 T318 S339 S380 S392 T393 T407 S428 T519 Y24		Aminoacyl tRNA synthetase signature: V73-A82, K305-N315 (p<1.1e-3) Cysteinylyl-tRNA synthetase signature: R66-A77, I103-V112, E230 C248, D261-E282, Y67-E282, H76-A494	putative cysteinylyl- tRNA synthetase [S. pombe] g1044932	BLAST GenBank BLIMPS-BLOCKS BLIMPS-PRINTS BLAST-PRODOM BLAST-DOMO MOTIFS
13	463	S54 S79 S124 S235 T253 T295 T411 T15 S366 Y84	N52	Succinyl CoA ligase family: I97-F111, V181-E193, L314-A343, L53-D367, D290-K442, L53-I463	ATP-specific succinyl-CoA synthetase beta subunit [Homo sapiens] g3766196	BLAST-GenBank BLIMPS-BLOCKS BLAST-PRODOM BLAST-DOMO MOTIFS